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<b>(21) International Application Number:</b> PCT/US98/27137 <b>(22) International Filing Date:</b> 18 December 1998 (18.12.98) <b>(30) Priority Data:</b> 08/994,825 19 December 1997 (19.12.97) US <b>(71) Applicant (for all designated States except US):</b> HESKA CORPORATION [US/US]; 1613 Prospect Parkway, Fort Collins, CO 80525 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MILHAUSEN, Michael, James [US/US]; 2292 Holyoke Drive, Boulder, CO 80303 (US). LUTZ, Susan, Bektesh [US/US]; 206 W. 12th Street, P.O. Box 417, New England, ND 58647-7210 (US). NG, Ray, K. [US/US]; 412 Alpert Circle, Fort Collins, CO 80525 (US). <b>(74) Agents:</b> HANLEY, Elizabeth, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF <b>(57) Abstract</b> <p>The present invention relates to immunogenic <i>Toxoplasma gondii</i> proteins, to <i>T. gondii</i> nucleic acid molecules, including those that encode such proteins and to antibodies raised against such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules and antibodies. Also included in the present invention are compositions comprising such proteins, nucleic acid molecules and/or antibodies, as well as the use of such compositions to inhibit oocyst shedding by cats due to infection with <i>T. gondii</i>. The present invention also includes the use of certain <i>T. gondii</i>-based antisera to identify such nucleic acid molecules and proteins, as well as nucleic acid molecules and proteins identified by such methods. The present invention also relates to methods for the detection of cysts and oocysts.</p>		

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## TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF

## FIELD OF THE INVENTION

The present invention relates to *Toxoplasma gondii* nucleic acid molecules,  
5 proteins encoded by such nucleic acid molecules, antibodies raised against such proteins  
and methods to identify such nucleic acid molecules, proteins or antibodies. The present  
invention also includes compositions comprising such nucleic acid molecules, proteins  
and antibodies, as well as their use for inhibiting oocyst shedding by cats infected with *T.*  
*gondii* and for protecting animals from diseases caused by *T. gondii*.

## 10 BACKGROUND OF THE INVENTION

Various attempts to develop a vaccine to both the asexual systemic stage and the  
sexual entero-epithelial stage of the *Toxoplasma* life cycle have been reported over the  
last thirty years (Hermentin, K. and Aspöck, H. (1988), *Zbl. Bakt. Hyg. A*, 269:423-436).  
These attempts can be grouped into the following categories: 1) immunization with  
15 whole killed organism, 2) immunization with selected antigens, either purified native or  
recombinant protein, 3) immunization with attenuated strains, and 4) immunization with  
irradiated organisms. Little success has been achieved with immunizations using whole  
killed organism (Frenkel, J.K. and Smith, D.D. (1982), *Journal of Parasitology*, 68:744-  
748). Partial success has been observed with the pure native protein P30 (Bulow, R.,  
20 and Boothroyd, J. C. (1991), *J. Immunol.* 147:3496) and with selected fractions of  
parasite lysates (Lunden, A. Lovgren, K. Uggla, A., and Araujo, F.G.; (1993) *Infection  
and Immunity*, 61: 2639-2643). However, attempts with purified recombinant antigens  
have not been successful (Lunden, A., Parmley, S.F., Bengtsson, K.L. and Araujo, F.G.  
(1997) *Parasitology Research*, 83:6-9). Studies with irradiated organisms have reported  
25 0-90% protection and are complicated by the uncertainty of truly inactivated irradiated  
preparations. Effective vaccines have been produced using attenuated strains. Two  
such mutant strains, ts-4 (Waldeland, H., Pfefferkorn, E.R., and Frenkel, J.K. (1983),  
*Journal of Parasitology*, 69:171-175) and S48 (Hartley, W.J. and Marshall, S.C. (1957),  
*New Zealand Veterinary Journal*, 5:119-124), successfully protect animals against the  
30 asexual systemic disease. These strains are delivered in the tachyzoite form and do not

protect cats from oocyst shedding. Another strain, T-263 (Frenkel, J.K.; Pfefferkorn, E.R.; Smith, D.D.; and Fishback, J.L. (1991), *American Journal of Veterinary Research*, 52:759-763) is an oocyst minus strain, but was shown to progress through most of the entero-epithelial stages in the cat intestine. Exposure to this strain induces immunity in the cat to oocyst shedding upon subsequent challenge. There remains a need for an effective vaccine for prevention of the diseases caused by infection with *Toxoplasma gondii*.

### SUMMARY OF THE INVENTION

The present invention relates to novel compositions and methods to inhibit *Toxoplasma gondii* (*T. gondii*) oocyst shedding by cats, thereby preventing the spread of *T. gondii* infection. According to the present invention there are provided isolated immunogenic *T. gondii* proteins and mimetopes thereof; *T. gondii* nucleic acid molecules, including those that encode such proteins; recombinant molecules including such nucleic acid molecules; recombinant viruses including such nucleic acid molecules; recombinant cells including such nucleic acid molecules; and antibodies that selectively bind to such immunogenic *T. gondii* proteins.

The present invention also includes methods to obtain and/or identify proteins, nucleic acid molecules, recombinant molecules, recombinant viruses, recombinant cells, and antibodies of the present invention. Also included are compositions comprising such proteins, nucleic acid molecules, recombinant molecules, recombinant viruses, recombinant cells, and antibodies, as well as use of such compositions to inhibit *T. gondii* oocyst shedding by cats infected with *T. gondii*, or for preventing *T. gondii* infection in an animal.

The present invention further includes the use of the nucleic acid molecules or proteins of the present invention as diagnostic reagents for the detection of *T. gondii* infection. In a preferred embodiment, the present invention includes a novel detection method and kit for detecting *T. gondii* oocysts in the feces of *T. gondii* infected cats.

One embodiment of the present invention is an isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein that can be identified by a method that includes the steps of: a) immunoscreening a *T. gondii* genomic expression library or



cDNA expression library with an antiserum, including an antiserum derived from intestinal secretions; and b) identifying a nucleic acid molecule in the library that expresses a protein that selectively binds to an antibody in the antiserum. Antisera to be used for screening include antiserum raised against *T. gondii* oocysts, antiserum raised  
5 against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection. Another embodiment is an isolated immunogenic *T. gondii* protein that can be identified by a method that includes the steps of: a) immunoscreening a *T. gondii* genomic expression library or cDNA expression library with such an antiserum; and b) identifying a protein expressed by the  
10 library that selectively binds to antibodies in the antiserum. Also included are methods to identify and isolate such nucleic acid molecules and proteins.

The present application also includes an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene that includes a nucleic acid sequence cited in Table 1. Also included in the present invention is an isolated  
15 nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene that includes a nucleic acid molecule cited in Table 1. Preferred nucleic acid molecules encode immunogenic *T. gondii* proteins. More preferred nucleic acid molecules are those cited in Table 1.

The present invention also relates to recombinant molecules, recombinant viruses  
20 and recombinant cells that include an isolated nucleic acid molecule of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

Another embodiment of the present invention is an isolated immunogenic protein  
25 encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene (i.e., with either the coding strand or the non-coding strand) comprising a nucleic acid sequence cited in Table 1 and/or a nucleic acid molecule cited in Table 1. Note that the nucleic acid molecule hybridizes with the non-coding strand of the gene, that is, with the complement of the coding strand of the gene. A preferred protein is an immunogenic *T. gondii* protein. More preferred proteins are those encoded

by nucleic acid molecules cited in Table 1. Also preferred are the proteins cited in Table 1.

The present invention also relates to: mimetopes of immunogenic *T. gondii* proteins and isolated antibodies that selectively bind to immunogenic *T. gondii* proteins or mimetopes thereof. Also included are methods, including recombinant methods, to  
5 produce proteins, mimetopes and antibodies of the present invention.

Yet another embodiment of the present invention is a composition to inhibit *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*. Such a composition includes one or more of the following protective compounds: an isolated immunogenic *T. gondii* protein encoded by a nucleic acid molecule that hybridizes under stringent  
10 hybridization conditions with a gene comprising a nucleic acid sequence cited in Table 1, and specifically with the non-coding-strand of that gene; an isolated antibody that selectively binds to said immunogenic *T. gondii* protein; and an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence cited in Table 1. Such a composition can also include an  
15 excipient, adjuvant or carrier. Preferred compositions comprising a nucleic acid molecule of the present invention include genetic vaccines, recombinant virus vaccines and recombinant cell vaccines. Also included in the present invention is a method to protect an animal, including a human, from disease caused by *T. gondii*, comprising the step of administering to the animal a composition of the present invention. Preferred  
20 animals to treat are cats in order to prevent oocyst shedding caused by *T. gondii* infection.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated immunogenic *T. gondii* proteins, isolated *T. gondii* nucleic acid molecules including those encoding such *T. gondii*  
25 proteins, recombinant molecules comprising such nucleic acid molecules, recombinant viruses comprising such nucleic acid molecules, cells transformed with such nucleic acid molecules (i.e., recombinant cells), and antibodies that selectively bind to immunogenic *T. gondii* proteins. As used herein, the terms isolated immunogenic *T. gondii* protein and isolated nucleic acid molecule refer to an immunogenic *T. gondii* protein and a *T.*  
30 *gondii* nucleic acid molecule, respectively, derived from *T. gondii* which can be obtained from its natural source or can be produced using, for example, recombinant nucleic acid

technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, and antibodies as compositions to protect animals from diseases caused by *T. gondii* and to inhibit *T. gondii* oocyst shedding in cats. As used herein, a cat refers to any member of the cat family (i.e., Felidae), including domestic cats, wild cats and zoo cats. Examples of cats include, but are not limited to, domestic cats, lions, tigers, leopards, panthers, cougars, bobcats, lynx, jaguars, cheetahs, and servals. A preferred cat to protect is a domestic cat. Further included in the present invention is the use of these proteins, nucleic acid molecules and antibodies for the detection of *T. gondii* infection in an animal or as targets for the development of chemotherapeutic agents against parasitic infection.

Immunogenic *T. gondii* protein and nucleic acid molecules of the present invention have utility because they represent novel targets for anti-parasite vaccines or chemotherapeutic agents. Compositions of the present invention can also be used as reagents for the diagnosis of *T. gondii* infection in cats and other animals, including humans. The products and processes of the present invention are advantageous because they enable the inhibition of *T. gondii* oocyst shedding in cats, the definitive hosts for *T. gondii* (i.e., the animals in which *T. gondii* reproduction takes place). It is to be noted that the proteins and nucleic acid molecules of the present invention have uses beyond eliciting an immune response despite denoting proteins of the present invention as immunogenic proteins.

As described in more detail in the Examples, it was very difficult to isolate a nucleic acid molecule encoding an immunogenic *T. gondii* protein selectively bound by antisera directed against *T. gondii* intestinal stages. Such stages are preferred because they represent the sexual cycle of *T. gondii*, the preferred target for development of a composition to inhibit oocyst shedding. Unfortunately, however, the *T. gondii* sexual cycle cannot currently be reproduced in culture, and, there is not a simple method by which to produce a cDNA (i.e., complementary DNA) library containing only *T. gondii* nucleic acid molecules of various stages of the sexual cycle. For example, the infected cat gut is the source of many of the sexual stages of *T. gondii*, and, as such, material to be used in identifying *T. gondii* immunogenic proteins are contaminated with cat

material. The present invention describes the development of new techniques to isolate and identify nucleic acid molecules encoding immunogenic *T. gondii* proteins. These techniques include (a) the isolation and enrichment of antisera against a variety of *T. gondii* life stages, several of which are only present in infected cats, at least

- 5 predominantly in infected cat guts, and (b) the use of such antisera to screen cDNA and genomic expression libraries to identify nucleic acid molecules that express *T. gondii* proteins that selectively bind to such antisera.

One embodiment of the present invention is an isolated protein that includes an immunogenic *T. gondii* protein. It is to be noted that the term "a" or "an" entity refers to  
10 one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. The terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein is a protein that has been removed from its natural  
15 milieu. The terms "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

An isolated protein of the present invention, including a homolog, can be  
20 identified in a straight-forward manner by the protein's ability to elicit an immune response against a naturally occurring *T. gondii* protein. Examples of *T. gondii* immunogenic proteins include proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation,  
25 prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of eliciting an immune response against a *T. gondii* immunogenic protein, and/or of binding to an antibody directed against a *T. gondii* immunogenic protein. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in  
30 the art, the animal will produce an immune response against at least one epitope of a *T.*

*gondii* immunogenic protein. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art. As used herein, the term "epitope" refers to the smallest portion of a protein or other antigen capable of selectively binding to the antigen binding site of an antibody or a T-cell receptor. It is well accepted by those skilled in the art that the minimal size of a protein epitope is about four to six amino acids. As is appreciated by those skilled in the art, an epitope can include amino acids that naturally are contiguous to each other as well as amino acids that, due to the tertiary structure of the natural protein, are in sufficiently close proximity to form an epitope. According to the present invention, an epitope includes a portion of a protein comprising at least about 4 amino acids, at least about 5 amino acids, at least about 6 amino acids, at least about 10 amino acids, at least about 15 amino acids, at least about 20 amino acids, at least about 25 amino acids, at least about 30 amino acids, at least about 35 amino acids, at least about 40 amino acids, at least about 50 amino acids, at least about 100 amino acids, at least about 150 amino acids, at least about 200 amino acids, at least about 250 amino acids, or at least about 300 amino acids.

Immunogenic *T. gondii* protein homologs can be the result of natural allelic variation or natural mutation. Immunogenic *T. gondii* protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

As used herein, a nucleic acid molecule encoding an immunogenic *T. gondii* protein includes nucleic acid sequences related to a natural *T. gondii* gene. As used herein, a *T. gondii* gene includes all regions of the genome related to the gene, such as regulatory regions that control production of the immunogenic *T. gondii* protein encoded by the gene (for example, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that

translates into a protein. A full-length coding region is that coding region that is translated into a full-length protein, i.e., a complete protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, a *T. gondii* gene of the present invention includes at least  
5 one of the nucleic acid molecules cited in Table 1 (i.e., the cited nucleic acid molecules).  
The coding strands of the cited nucleic acid molecules are represented, respectively, by  
the nucleic acid sequences (i.e., the cited nucleic acid sequences) shown in Table 1.  
Also presented in Table 1 are the deduced amino acid sequences encoded by each of the  
cited nucleic acid molecules (i.e., the cited amino acid sequences) and the protein name  
10 designations (i.e., the cited proteins).

Table 1

-9-

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
1	DNA	nTG1 <sub>357</sub>		Tg-41
2	Protein		PTG1 <sub>119</sub>	PTG-41
3	DNA	nTG2 <sub>339</sub>		Tg-45
4	Protein		PTG2 <sub>108</sub>	PTG-45
5	DNA	nTG4 <sub>526</sub>		Tg-50
6	Protein		PTG4 <sub>175</sub>	PTG-50
7	cDNA	nTG4 <sub>1478</sub>		Tg-50c
8	Protein		PTG4 <sub>381</sub>	PTG-50c
9	DNA	nTG5 <sub>657</sub>		Q2-4
10	Protein		PTG5 <sub>219</sub>	PQ2-4
11	cDNA	nTG5 <sub>1029</sub>		Q2-4c
12	Protein		PTG5 <sub>273</sub>	PQ2-4c
13	DNA	nTG6 <sub>425</sub>		Q2-9
14	Protein		PTG6 <sub>142</sub>	PQ2-9
15	DNA	nTG7 <sub>417</sub>		Q2-10
16	Protein		PTG7 <sub>139</sub>	PQ2-10
17	DNA	nTG8 <sub>507</sub>		Q2-11
18	Protein		PTG8 <sub>51</sub>	PQ2-11
19	DNA	nTG9 <sub>718</sub>		4499-9
20	Protein		PTG9 <sub>99</sub>	P4499-9
21	DNA	nTG10 <sub>441</sub>		4604-2
22	Protein		PTG10 <sub>147</sub>	P4604-2
23	DNA	nTG11 <sub>428</sub>		4604-3
24	Protein		PTG11 <sub>134</sub>	P4604-3
25	DNA	nTG13 <sub>282</sub>		4604-5
26	DNA	nTG15 <sub>304</sub>		4604-10
27	Protein		PTG15 <sub>101</sub>	P4604-10
28	DNA	nTG16 <sub>284</sub>		4604-17
29	Protein		PTG16 <sub>95</sub>	P4604-17
30	DNA	nTG17 <sub>690</sub>		4604-54
31	Protein		PTG17 <sub>230</sub>	P4604-54
32	DNA	nTG18 <sub>313</sub>		4604-62
33	Protein		PTG18 <sub>54</sub>	P4604-62
34	DNA	nTG19 <sub>389</sub>		4604-63
35	Protein		PTG19 <sub>65</sub>	P4604-63

Table 1

-10-

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
36	DNA	nTG21 <sub>548</sub>		4604-69
37	Protein		PTG21 <sub>183</sub>	P4604-69
38	DNA	nTG22 <sub>310</sub>		BZ1-2
39	Protein		PTG22 <sub>95</sub>	PBZ1-2
40	DNA	nTG23 <sub>220</sub>		BZ1-3
41	Protein		PTG23 <sub>73</sub>	PBZ1-3
42	DNA	nTG24 <sub>642</sub>		BZ1-6
43	Protein		PTG24 <sub>34</sub>	PBZ1-6
44	DNA	nTG25 <sub>381</sub>		BZ2-3
45	Protein		PTG25 <sub>27</sub>	PBZ2-3
46	DNA	nTG26 <sub>432</sub>		BZ2-5
47	Protein		PTG26 <sub>85</sub>	PBZ2-5
48	DNA	nTG27 <sub>282</sub>		BZ3-2
49	Protein		PTG27 <sub>35</sub>	PBZ3-2
50	DNA	nTG28 <sub>466</sub>		BZ4-3
51	Protein		PTG28 <sub>71</sub>	PBZ4-3
52	DNA	nTG30 <sub>539</sub>		BZ4-6
53	Protein		PTG30 <sub>20</sub>	PBZ4-6
54	DNA	nTG31 <sub>1233</sub>		AMX/I-5
55	DNA	nTG32 <sub>411</sub>		AMX/I-6
56	Protein		PTG32 <sub>60</sub>	PAMX/I-6
57	DNA	nTG33 <sub>441</sub>		AMX/I-7
58	Protein		PTG33 <sub>118</sub>	PAMX/I-7
59	DNA	nTG34 <sub>491</sub>		AMX/I-9
60	Protein		PTG34 <sub>34</sub>	PAMX/I-9
61	DNA	nTG35 <sub>387</sub>		AMX/I-10
62	Protein		PTG35 <sub>129</sub>	PAMX/I-10
63	DNA	nTG36 <sub>417</sub>		AMI-23
64	Protein		PTG36 <sub>139</sub>	PAMI-23
65	DNA	nTG37 <sub>416</sub>		AMI-24
66	Protein		PTG37 <sub>138</sub>	PAMI-24
67	DNA	nTG38 <sub>500</sub>		AMI-28
68	DNA	nTG40 <sub>321</sub>		AMI-47
69	Protein		PTG40 <sub>73</sub>	PAMI-47
70	DNA	nTG41 <sub>513+C86</sub>		OC-1
71	Protein		PTG41 <sub>171</sub>	POC-1



Table 1

-11-

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
72	DNA	nTG42 <sub>528</sub>		OC-2
73	Protein		PTG42 <sub>176</sub>	POC-2
74	DNA	nTG43 <sub>375</sub>		OC-13
75	Protein		PTG43 <sub>125</sub>	POC-13
76	DNA	nTG44 <sub>543</sub>		OC-14
77	Protein		PTG44 <sub>89</sub>	POC-14
78	DNA	nTG45 <sub>573</sub>		OC-22
79	Protein		PTG45 <sub>191</sub>	POC-22
80	DNA	nTG46 <sub>1835</sub>		OC-23
81	Protein		PTG46 <sub>612</sub>	POC-23
82	DNA	nTG48 <sub>604</sub>		4CQA7f
83	Protein		PTG48 <sub>112</sub>	P4CQA7f
84	DNA	nTG48 <sub>549</sub>		4CQA7r
85	DNA	nTG49 <sub>270</sub>		4CQA11
86	Protein		PTG49 <sub>90</sub>	P4CQA11
87	DNA	nTG50 <sub>306</sub>		4CQA19
88	Protein		PTG50 <sub>102</sub>	P4CQA19
89	DNA	nTG51 <sub>804</sub>		4CQA21
90	Protein		PTG51 <sub>268</sub>	P4CQA21
91	DNA	nTG52 <sub>867</sub>		4CQA22
92	Protein		PTG52 <sub>289</sub>	P4CQA22
93	DNA	nTG53 <sub>1434</sub>		4CQA24
94	Protein		PTG53 <sub>164</sub>	P4CQA24
95	DNA	nTG54 <sub>680</sub>		4CQA25
96	Protein		PTG54 <sub>227</sub>	P4CQA25
97	DNA	nTG55 <sub>296</sub>		4CQA26
98	Protein		P1G55 <sub>99</sub>	P4CQA26
99	DNA	nTG56 <sub>723</sub>		4CQA27
100	Protein		PTG56 <sub>53</sub>	P4CQA27
101	DNA	nTG57 <sub>270</sub>		4CQA29
102	Protein		PTG57 <sub>90</sub>	P4CQA29
103	DNA	nTG58 <sub>503</sub>		R8050-2
104	Protein		PTG58 <sub>82</sub>	PR8050-2
105	DNA	nTG60 <sub>322</sub>		R8050-5
106	Protein		PTG60 <sub>73</sub>	PR8050-5

Table 1

-12-

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
107	DNA	nTG61 <sub>390</sub>		R8050-6
108	Protein		PTG61 <sub>67</sub>	PR8050-6
109	DNA	nTG62 <sub>699</sub>		M2A1
110	Protein		PTG62 <sub>233</sub>	PM2A1
111	DNA	nTG63 <sub>419</sub>		M2A2
112	Protein		PTG63 <sub>140</sub>	PM2A2
113	DNA	nTG64 <sub>303</sub>		M2A3
114	Protein		PTG64 <sub>101</sub>	PM2A3
115	DNA	nTG65 <sub>696</sub>		M2A4
116	Protein		PTG65 <sub>232</sub>	PM2A4
117	DNA	nTG66 <sub>173</sub>		M2A5
118	Protein		PTG66 <sub>58</sub>	PM2A5
119	DNA	nTG67 <sub>369</sub>		M2A6
120	Protein		PTG67 <sub>123</sub>	PM2A6
121	DNA	nTG68 <sub>566</sub>		M2A7
122	Protein		PTG68 <sub>61</sub>	PM2A7
123	DNA	nTG69 <sub>816</sub>		M2A11
124	Protein		PTG69 <sub>205</sub>	PM2A11
125	DNA	nTG70 <sub>762</sub>		M2A16
126	Protein		PTG70 <sub>254</sub>	PM2A16
127	DNA	nTG71 <sub>236</sub>		M2A18
128	Protein		PTG71 <sub>79</sub>	PM2A18
129	DNA	nTG72 <sub>569</sub>		M2A19
130	Protein		PTG72 <sub>190</sub>	PM2A19
131	DNA	nTG73 <sub>232</sub>		M2A20
132	DNA	nTG74 <sub>276</sub>		M2A21
133	Protein		PTG74 <sub>92</sub>	PM2A21
134	DNA	nTG75 <sub>309</sub>		M2A22
135	Protein		PTG75 <sub>103</sub>	PM2A22
136	DNA	nTG76 <sub>534</sub>		M2A23
137	Protein		PTG76 <sub>178</sub>	PM2A23
138	DNA	nTG76 <sub>423</sub>		M2A23
139	DNA	nTG77 <sub>327</sub>		M2A24
140	Protein		PTG77 <sub>109</sub>	PM2A24
141	DNA	nTG78 <sub>444</sub>		M2A25
142	Protein		PTG78 <sub>148</sub>	PM2A25

Table 1

-13-

SEQ ID NO	TYPE	Nucleic Acid Molecules	Amino Acid Molecules	Original Designation
143	DNA	nTG79 <sub>928</sub>		M2A29
144	Protein		PTG79 <sub>19</sub>	PM2A29
265	DNA	nTG22 <sub>310a</sub>		BZ1-2-a
266	Protein		PTG22 <sub>95a</sub>	PBZ1-2-a
267	DNA	nTG64 <sub>303a</sub>		M2A3-a
268	Protein		PTG64 <sub>101a</sub>	PM2A3-a
269	DNA	nTG71 <sub>236a</sub>		M2A18-a
270	Protein		PTG71 <sub>79a</sub>	PM2A18-a
271	DNA	nTG6 <sub>425a</sub>		Q2-9-1-a
272	Protein		PTG6 <sub>142a</sub>	PQ2-9-a
273	DNA	nTG41 <sub>513a</sub>		OC-1-a
274	Protein		PTG41 <sub>171a</sub>	POC-1-a
282	cDNA	nTG <sub>1225</sub>		MGIS42
283	Protein		PTG <sub>28</sub>	PMGIS42
284	DNA	nTG <sub>1225</sub>		rc
292	cDNA	nTG <sub>1573</sub>		MGIS44
293	Protein		PTG <sub>73</sub>	PMGIS44
294	DNA	nTG <sub>1573</sub>		rc
306	cDNA	nTG <sub>2417</sub>		MGIS48
307	Protein		PTG <sub>9</sub>	PMGIS48
308	DNA	nTG <sub>2417</sub>		rc
311	cDNA	nTG <sub>1785</sub>		MGIS65
312	Protein		PTG <sub>24</sub>	PMGIS65
313	DNA	nTG <sub>1785</sub>		rc
338	DNA	nTG <sub>647</sub>		511-44 genomic
339	DNA	nTG <sub>647</sub>		rc
340	cDNA	nTG <sub>867</sub>		511-44 coding region
341	Protein		PTG <sub>288</sub>	P511-44
342	DNA	nTG <sub>867</sub>		rc
343	cDNA	nTG <sub>1397</sub>		511-44cDNA
345	DNA	nTG <sub>1397</sub>		rc

It should be noted that because nucleic acid sequencing technology is not entirely error-free, the nucleic acid sequences disclosed in the present invention (as well as other nucleic acid and protein sequences presented herein) represent the apparent nucleic acid sequences of the nucleic acid molecules encoding *T. gondii* proteins of the present invention. The nucleic acid molecules cited in Table 1 also include the complementary (i.e., apparently non-coding) strands. As used herein the terms "complementary strand" and "complement" refer to the nucleic acid sequence of the DNA strand that is fully complementary to the DNA strand having the listed sequence, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to (i.e., can form a complete double helix with) the strand for which the sequence is cited. Production of the cited nucleic acid molecules is disclosed in the Examples as are methods to obtain nucleic acid sequences of the coding strands of such molecules and the amino acid sequences deduced therefrom.

In another embodiment, a *T. gondii* gene or nucleic acid molecule can be a naturally occurring allelic variant that includes a similar but not identical sequence to the cited nucleic acid molecules. A naturally occurring allelic variant of a *T. gondii* gene including any of the above-listed nucleic acid sequences is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including at least one of the above-listed sequences, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given *T. gondii* organism or population, because, for example, the genome goes through a diploid stage, and sexual reproduction results in the reassortment of alleles.

In one embodiment of the present invention, an isolated immunogenic *T. gondii* protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene encoding an immunogenic *T. gondii* protein. The minimal size of a *T. gondii* protein of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridizing under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the percent homology between the *T. gondii* nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered (i.e., localized) in distinct regions on a given nucleic acid molecule.

The minimal size of a nucleic acid molecule capable of forming a stable hybrid with a gene encoding an immunogenic *T. gondii* protein is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if it is AT-rich. The minimal size of a nucleic acid molecule used to encode an immunogenic *T. gondii* protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an immunogenic *T. gondii* protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention because a nucleic acid molecule of the present invention can include a portion of a gene, an entire gene, or multiple genes. A preferred nucleic acid molecule of the present invention is a nucleic acid molecule that is at least 12 nucleotides in length. Also preferred are nucleic acid molecules that are at least 18 nucleotides, or at least 20 nucleotides, or at least 25 nucleotides, or at least 30 nucleotides, or at least 40 nucleotides, or at least 50 nucleotides, or at least 70 nucleotides, or at least 100 nucleotides, or at least 150 nucleotides, or at least 200 nucleotides, or at least 250 nucleotides, or at least 300 nucleotides, or at least 350 nucleotides, or at least 400 nucleotides, or at least 500 nucleotides, or at least 750

nucleotides, or at least 1000 nucleotides, or at least 1500 nucleotides, or at least 1750 nucleotides, or at least 2000 nucleotides, or at least 2250 nucleotides, or at least 2417 nucleotides in length. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or  
5 functional portion of such a protein is desired.

Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities  
10 between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal. Biochem.* 138, 267-284, each of which is incorporated by reference herein in its entirety. As explained in detail in the cited references, the determination of hybridization  
15 conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about  
20 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or  $T_m$ , of a given nucleic acid molecule. As defined in the formula below,  $T_m$  is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

25 
$$T_m = 81.5^{\circ}\text{C} + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61(\%\text{formamide}).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature ( $T_d$ ), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

30 
$$T_d = 4(G + C) + 2(A + T).$$

A temperature of 5°C below  $T_d$  is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base-pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect  $T_m$  or  $T_d$  for nucleic acid molecules of different sizes. For example,  $T_m$  decreases about 1°C for each 1% of mismatched base-pairs for hybrids greater than about 150 bp, and  $T_d$  decreases about 5°C for each mismatched base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with less than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow hybridization between molecules having about 30% or less base-pair mismatch (i.e., about 70% or greater identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures.

Typically, the actual hybridization reaction is done under non-stringent conditions, i.e.,

at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

For example, if the skilled artisan wished to identify a nucleic acid molecule that  
5 hybridizes under stringent hybridization conditions with a *T. gondii* nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. As an example, the average G + C content of *Dirofilaria immitis* DNA is about 35%. The unknown nucleic acid molecules would be attached to a support membrane, and the 150 bp probe would be labeled, e.g. with a radioactive tag. The hybridization reaction  
10 could be carried out in a solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency  
15 hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the  $T_m$  of perfect hybrids would be about 79°C:

$$81.5^{\circ}\text{C} + 16.6 \log (.15\text{M}) + (0.41 \times 35) - (500/150) - (0.61 \times 0) = 79^{\circ}\text{C}.$$

Thus, to achieve hybridization with nucleic acid molecules having about 30% base-pair  
20 mismatch, hybridization washes would be carried out at a temperature of about 49°C. It is thus within the skill of one in the art to calculate additional hybridization temperatures based on the desired percentage base-pair mismatch, formulae and G/C content disclosed herein. For example, it is appreciated by one skilled in the art that as the nucleic acid molecule to be tested for hybridization against nucleic acid molecules of the present  
25 invention having sequences specified herein becomes longer than 150 nucleotides, the  $T_m$  for a hybridization reaction allowing up to 30% base-pair mismatch will not vary significantly from 49°C.

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid  
30 sequences. These computer programs include various known methods to determine the



percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, GCG™ (available from Genetics Computer Group, Madison, WI), DNAsis™ (available from Hitachi Software, San Bruno, CA) and MacVector™ (available from the Eastman Kodak Company, New Haven, CT). A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the GCG™ program, Bestfit function with default parameter settings, or a gap weight of 12, a length weight of 4, an average match of 2.912, and an average mismatch of -2.003.

A preferred immunogenic *T. gondii* protein of the present invention is a compound that, when administered to an animal in an effective manner, is capable of protecting that animal from disease caused by *T. gondii* or, in the case of cats, is capable of preventing *T. gondii* oocyst shedding in cats infected with *T. gondii*. In accordance with the present invention, the ability of an immunogenic *T. gondii* protein of the present invention to protect an animal from *T. gondii* disease refers to the ability of that protein to, for example, treat, ameliorate and/or prevent disease caused by *T. gondii*. In one embodiment, an immunogenic *T. gondii* protein of the present invention can elicit an immune response (including a humoral and/or cellular immune response) against *T. gondii*.

The present invention also includes mimetopes of immunogenic *T. gondii* proteins of the present invention. As used herein, a mimetope of an immunogenic *T. gondii* protein of the present invention refers to any compound that is able to mimic the activity of such an immunogenic *T. gondii* protein, often because the mimetope has a structure that mimics the particular *T. gondii* protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and synthetic or natural organic molecules, including nucleic

acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding  
5 binding partner.

One embodiment of an immunogenic *T. gondii* protein of the present invention is a fusion protein that includes an immunogenic *T. gondii* protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's  
10 stability; act as an immunopotentiator to enhance an immune response against an immunogenic *T. gondii* protein; and/or assist in purification of an immunogenic *T. gondii* protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein).

15 Fusion segments can be joined to amino and/or carboxyl termini of the immunogenic *T. gondii* protein-containing domain of the protein and can be susceptible to cleavage in order to enable straightforward recovery of an immunogenic *T. gondii* protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a nucleic acid molecule that encodes a protein including the fusion segment attached to  
20 either the carboxyl and/or amino terminal end of an immunogenic *T. gondii* protein-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a  
25 portion of  $\beta$ -galactosidase, a strep tag peptide, a T7 tag peptide, a Flag™ peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide.

30 In another embodiment, an immunogenic *T. gondii* protein of the present invention also includes at least one additional protein segment that is capable of

- protecting an animal from one or more diseases. Such a multivalent protective protein can be produced, for example, by culturing a cell transformed with a nucleic acid molecule comprising two or more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent protective
- 5 compound containing at least two protective compounds capable of protecting an animal from diseases caused, for example, by at least one infectious agent.

Examples of multivalent protective compounds include, but are not limited to, an immunogenic *T. gondii* protein of the present invention attached to one or more compounds protective against one or more other infectious agents, particularly an agent

10 that infects cats. In another embodiment, one or more protective compounds can be included in a multivalent vaccine comprising an immunogenic *T. gondii* protein of the present invention and one or more other protective molecules as separate compounds.

- A preferred isolated immunogenic *T. gondii* protein of the present invention includes a protein that is encoded by a nucleic acid molecule that hybridizes under
- 15 stringent hybridization conditions with a gene (i.e., with the non-coding strand which is a complement of the coding strand) comprising at least one of the nucleic acid molecules cited in Table 1. As such, also preferred is a protein that is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the non-coding strand of a gene comprising at least one of the nucleic acid sequences cited in Table 1.
- 20 More preferred is a protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid molecules particularly since those nucleic acid molecules have been shown to encode proteins that selectively bind to antiserum that either was raised against *T. gondii* oocysts, bradyzoites, or infected cat gut, or was isolated from a cat immune to *T. gondii* infection.
- 25 As such, also preferred is a protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with the complement of at least one of the cited nucleic acid sequences.

- Even more preferred are isolated proteins having an amino acid sequence encoded by a nucleic acid molecules that are at least about 75%, preferably at least about
- 30 80%, more preferably at least about 85%. even more preferably at least about 90%, even more preferably at least about 95%, and even more preferably at least about 98%

identical to one of the nucleic acid molecules and/or nucleic acid sequences cited in Table 1. Also preferred are proteins that comprise one or more epitopes of any of the proteins having such amino acid sequences.

A particularly preferred isolated protein of the present invention is a protein  
5 having an amino acid sequence encoded by at least one of the cited nucleic acid molecules and/or cited nucleic acid sequences, a protein encoded by an allelic variant of at least one of the cited nucleic acid molecules and/or nucleic acid sequences, or a protein comprising an epitope of any of the proteins having such amino acid sequences.

In one embodiment, preferred immunogenic *T. gondii* proteins of the present  
10 invention include proteins that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the proteins cited in Table 1. As such, also preferred are proteins that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%,  
15 and even more preferably at least about 95% identical to at least one of the amino acid sequences cited in Table 1. Also preferred are proteins that comprise one or more epitopes of any of such proteins. More preferred are immunogenic *T. gondii* proteins comprising the cited proteins and/or having the cited amino acid sequences, proteins encoded by allelic variants of nucleic acid molecules encoding proteins including the  
20 cited proteins and/or having the cited amino acid sequences, and proteins having one or more epitopes of such proteins.

Another embodiment of the present invention is an isolated nucleic acid molecule comprising a *T. gondii* nucleic acid molecule that encodes an immunogenic *T. gondii* protein. The identifying characteristics of such nucleic acid molecules are heretofore  
25 described. A nucleic acid molecule of the present invention can include an isolated natural *T. gondii* nucleic acid molecule or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of the present invention can include one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present  
30 invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another

nucleic acid molecule. The minimal size of an *T. gondii* nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been  
5 subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. Accordingly, the term "isolated", as used herein to describe a nucleic acid molecule, does not reflect the extent to which the nucleic acid molecule has been purified. An isolated *T. gondii* nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant nucleic acid technology  
10 (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated *T. gondii* nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an immunogenic  
15 *T. gondii* protein of the present invention.

A homolog of a nucleic acid molecule encoding an immunogenic *T. gondii* protein can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Sambrook et al., *ibid.*, is incorporated by reference  
20 herein in its entirety. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic  
25 acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a nucleic acid molecule encoding an immunogenic *T. gondii* protein or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of an immunogenic *T. gondii* protein).

30 An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one immunogenic *T. gondii* protein of the present

invention, examples of which are disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a  
5 nucleic acid molecule, or a nucleic acid sequence, capable of encoding an *T. gondii* protein.

A preferred nucleic acid molecule of the present invention, when administered to a cat, is capable of preventing *T. gondii* oocyst shedding. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a  
10 molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode a protective protein (e.g., an immunogenic *T. gondii* protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e., as a genetic vaccine) or in a vehicle such as a recombinant virus  
15 vaccine or a recombinant cell vaccine. Another preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of preventing disease in that animal caused by *T. gondii*.

One embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule  
20 comprising at least one of the nucleic acid molecules cited in Table 1. As such, also preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the nucleic acid sequences cited in Table 1 or with a complement of such a sequence. More preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic  
25 acid molecules. As such, also preferred is a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the cited nucleic acid sequences or with a complement thereof.

Even more preferred are isolated nucleic acid molecules that are at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more  
30 preferably at least about 90%, even more preferably at least about 95%, and even more preferably at least about 98% identical to one of the nucleic acid molecules and/or

nucleic acid sequences cited in Table 1. Also preferred are nucleic acid molecules that form stable hybrids with nucleic acid molecules having those percent identities.

A particularly preferred isolated nucleic acid molecule of the present invention is a nucleic acid molecule that comprises at least one of the cited nucleic acid molecules  
5 and/or cited nucleic acid sequences, a nucleic acid molecule that is an allelic variant of at least one of the cited nucleic acid molecules and/or nucleic acid sequences, or a nucleic acid molecule that is a portion thereof (i.e., a nucleic acid molecule that forms a stable hybrid with at least one of the cited nucleic acid molecules or allelic variants thereof).

In one embodiment, a nucleic acid molecule encoding an immunogenic *T. gondii*  
10 protein of the present invention encodes a protein that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to the proteins cited in Table 1. Even more preferred is a nucleic acid molecule encoding a protein cited in Table 1 or an allelic variant of such a nucleic acid molecule. Also preferred are nucleic acid  
15 molecules encoding proteins comprising one or more epitopes of proteins having the cited percent identities or epitopes of proteins cited in Table 1 or encoded by nucleic acid molecules that are allelic variants of nucleic acid molecules cited in Table 1.

In another embodiment, a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention encodes a protein having an amino acid sequence  
20 that is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to at least one of the amino acid sequences cited in Table 1. Even more preferred is a nucleic acid molecule encoding a protein having an amino acid sequence cited in Table 1 or an allelic variant of such a nucleic acid molecule. Also preferred are  
25 nucleic acid molecules encoding proteins comprising one or more epitopes of proteins having the cited percent identities or epitopes of proteins having amino acid sequences cited in Table 1 or encoded by nucleic acid molecules that are allelic variants of nucleic acid molecules cited in Table 1.

Note that nucleic acid molecules of the present invention can include nucleotide  
30 sequences in addition to those disclosed above, such as, but not limited to, nucleotide sequences comprising a full-length gene, a full-length coding region, a nucleic acid

molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent protective compound. Also included in the present invention are nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed. Preferred nucleic acid molecules of the present invention include fragments of the nucleic acid molecules disclosed in Table 1.

Knowing the nucleic acid sequences of certain nucleic acid molecules encoding immunogenic *T. gondii* proteins of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other nucleic acid molecules encoding an immunogenic *T. gondii* proteins. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include *T. gondii* cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources from which to amplify nucleic acid molecules include *T. gondii* cDNA and genomic DNA. Techniques to clone and amplify nucleic acid molecules are disclosed, for example, in Sambrook et al., *ibid*.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising nucleic acid molecules encoding immunogenic *T. gondii* proteins. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of about 100 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic



acid molecules encoding immunogenic *T. gondii* proteins, primers to produce nucleic acid molecules encoding immunogenic *T. gondii* proteins, or reagents to inhibit immunogenic *T. gondii* protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also  
5 includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing compositions can be administered to an animal using techniques known to those skilled in the art.

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into  
10 any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or  
15 eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of nucleic acid molecule encoding immunogenic *T. gondii* proteins of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an  
20 expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also  
25 capable of replicating within the host cell. Expression vectors can be operative in either prokaryotic or eukaryotic cells, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can  
30 direct gene expression in bacterial, yeast, *T. gondii* and mammalian cells, and more preferably in the cell types disclosed herein.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth or other endoparasite, or insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, *rrnB*, bacteriophage lambda (such as lambda  $p_L$  and lambda  $p_R$  and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with *T. gondii*.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include those cited in Table 1. Particularly preferred recombinant molecules of the present invention

include those recombinant molecules, the production of which are described in the Examples section.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed *T. gondii* protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteasome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include nucleic acid molecules encoding immunogenic *T. gondii* proteins disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include those listed in Table 1.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing *T. gondii* proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, protozoan, helminth, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1<sub>x</sub>3987 and SR-11<sub>x</sub>4072; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK<sup>31</sup> cells and/or HeLa cells. In one embodiment, the proteins may be expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein. Particularly preferred recombinant cells  
5 include those recombinant cells, the production of which are disclosed in the Examples section.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including a nucleic acid molecule encoding at least one immunogenic *T. gondii* protein of the present invention and one or more other nucleic  
10 acid molecules encoding other protective compounds, as disclosed herein (e.g., to produce multivalent vaccines).

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies  
of the nucleic acid molecules within a host cell, the efficiency with which those nucleic  
15 acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more  
20 host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that  
25 destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated *T. gondii* proteins of the present invention can be produced in a variety  
30 of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an

isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an immunogenic *T. gondii* protein of the present invention. Effective media typically comprise an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Suitable culturing conditions are within the expertise of one of ordinary skill in the art. Examples of suitable conditions are included in the Examples section.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a composition to inhibit *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal, or as a diagnostic reagent. A composition for inhibiting *T. gondii* oocyst

shedding in a cat due to infection with *T. gondii* animals, or for preventing *T. gondii* infection in an animal for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to an immunogenic *T. gondii* protein of the present invention or a mimetope thereof (e.g., anti-*T. gondii* antibodies). As used herein, the term "selectively binds to" an immunogenic *T. gondii* protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated by reference herein in its entirety. An anti-*T. gondii* antibody of the present invention preferably selectively binds to an immunogenic *T. gondii* protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in any bodily fluid that has been collected (e.g., recovered) from an animal. Suitable bodily fluids include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Thus, serum containing antibodies (i.e., antiserum) or mucosal secretions, such as intestinal secretions, are examples of isolated antibodies. Other embodiments of antibodies include antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce *T. gondii* proteins of the present invention.

Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a composition for inhibiting *T. gondii* oocyst shedding in a cat due to infection  
5 with *T. gondii*, or for preventing *T. gondii* infection in an animal.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as compounds to passively immunize a cat in order to inhibit the cat from shedding *T. gondii* oocysts, (b) as reagents in assays to detect infection by *T. gondii* and/or (c) as  
10 tools to screen expression libraries and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants.

One embodiment of the present invention includes a method for identifying a nucleic acid molecule encoding an immunogenic *T. gondii* protein. According to this method, antiserum (comprising either monoclonal or polyclonal antibodies) raised  
15 against a *T. gondii* developmental stage or stages, or against oocysts, is used to immunoscreen a *T. gondii* genomic expression library or a *T. gondii* cDNA expression library, and a nucleic acid molecule expressing an immunogenic *T. gondii* protein is identified by its ability to selectively bind to at least one antibody within the antiserum. As used herein, the term immunoscreen refers to a method in which antibodies are mixed  
20 with a sample to determine whether the sample contains a substance to which the antibodies can selectively bind. A substance is identified by its ability to selectively bind to such antibodies. Although general methods to accomplishing immunoscreening of expression libraries are known to those skilled in the art, the exact method to use such a technique to identify *T. gondii* immunogenic proteins was not previously known. The  
25 present invention includes the identification of antisera that are useful in the identification and isolation of nucleic acid molecules encoding *T. gondii* immunogenic proteins. Such antisera include antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection. In one embodiment,  
30 antiserum as described above is enriched for antibodies specific to *T. gondii*



gametogenic stages. In a preferred embodiment, polyclonal antiserum is produced by exposing an animal to a *T. gondii* antigen or antigens, then isolating the antiserum from the animal so exposed. Methods to produce and use the various antisera are described in the Examples section.

- 5           In another embodiment, immunoscreening as described above can be used to identify an immunogenic *T. gondii* protein. According to this method, antiserum as described above is used to immunoscreen a *T. gondii* genomic expression library or cDNA expression library, and an immunogenic *T. gondii* protein is identified. *T. gondii* immunogenic proteins can also be identified by immunoscreening preparations  
10           containing *T. gondii* antigens (e.g., *T. gondii* oocysts, bradyzoites, infected cat guts) using antiserum as described above.

Nucleic acid molecules and proteins identified using such techniques can be isolated (i.e., recovered) and purified to a desired state of purity using techniques known to those skilled in the art.

- 15           One embodiment of the present invention is a composition that, when administered to a cat in an effective manner, is capable of preventing that cat from shedding *T. gondii* oocysts. Compositions of the present invention, useful for inhibiting *T. gondii* oocyst shedding in a cat due to infection with *T. gondii* (i.e., infection with *T. gondii* causes oocyst shedding in cats), include at least one of the following protective  
20           compounds: an isolated immunogenic *T. gondii* protein or a mimotope thereof, an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising one of the nucleic acid molecules and/or nucleic acid sequences cited in Table 1, an isolated antibody that selectively binds to an immunogenic *T. gondii* protein, an inhibitor of *T. gondii* function identified by its ability  
25           to bind to an immunogenic *T. gondii* protein and thereby impede development and/or the production of oocysts, or a mixture thereof (i.e., combination of at least two of the compounds). As used herein, a protective compound refers to a compound that, when administered to a cat in an effective manner, is able to inhibit the cat from shedding *T. gondii* oocysts upon infection with *T. gondii*. The term protective compound also refers  
30           to a compound that, when administered to a cat or other animal, including a human, in

an effective manner, is able to prevent or ameliorate disease caused by infection with *T. gondii*. Examples of proteins, nucleic acid molecules, antibodies and inhibitors of the present invention are disclosed herein.

The present invention also includes a composition comprising at least one *T. gondii* protein-based compound of the present invention in combination with at least one additional compound protective against one or more infectious agents. Examples of such compounds and infectious agents are disclosed herein.

Compositions of the present invention that are useful for preventing *T. gondii* infection can be administered to any animal susceptible to such therapy, preferably to mammals.

In order to inhibit a cat from shedding *T. gondii* oocysts, a composition of the present invention is administered to the cat in a manner effective to inhibit that cat from shedding *T. gondii* oocysts. In a preferred embodiment, compositions of the present invention are administered to cats prior to infection in order to prevent oocyst shedding (i.e., as a preventative vaccine). In another embodiment, compositions of the present invention can be administered to animals after infection in order to treat disease caused by *T. gondii* (e.g., as a therapeutic vaccine).

Compositions of the present invention, useful for inhibiting *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, or for preventing *T. gondii* infection in an animal, can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, — or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a

non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a composition useful for inhibiting oocyst shedding in a cat infected with *T. gondii*, or for preventing *T. gondii* infection in an animal, can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a composition useful for inhibiting oocyst shedding in a cat infected with *T. gondii*, or for preventing *T. gondii* infection in an animal, can include a carrier. Carriers include compounds that increase the half-life of a composition of the present invention in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain dose levels of the composition effective to either inhibit oocyst shedding by cats, or to protect an animal from disease caused by *T. gondii*. The composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

Compositions of the present invention can be administered to cats prior to infection in order to inhibit oocyst shedding, and/or can be administered to cats or other animals, including humans, before infection in order to prevent disease caused by *T. gondii* infection, or after infection in order to treat disease caused by *T. gondii*. For example, nucleic acid molecules, proteins, mimetopes thereof, antibodies thereof, and inhibitors thereof can be used to treat or prevent disease caused by *T. gondii* infection. Acceptable protocols to administer compositions of the present invention include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of protecting an animal from disease when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody composition of the present

invention is from about 1 microgram ( $\mu\text{g}$ ) to about 10 milligrams (mg) of the composition per kilogram body weight of the animal. Booster doses can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10  $\mu\text{g}$  to about 1 mg of the composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, injection, oral administration, inhalation, nasal administration, intraocular administration, anal administration, topical administration, particle bombardment, and intradermal scarification. Preferred injection methods include intradermal, intramuscular, subcutaneous, intravenous methods, with intradermal injection and intramuscular injection being more preferred. A particularly preferred method is mucosal administration.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein or protective RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a nucleic acid not packaged in a viral coat or cell as a genetic vaccine (e.g., as "naked" DNA or RNA molecules with or without a non-viral/non-cellular carrier (e.g., liposome, hydrogel, etc.) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A genetic vaccine of the present invention includes a recombinant molecule of the present invention. As such, a genetic vaccine comprises at least one isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein operatively linked to a eukaryotic or prokaryotic transcription control region. A genetic vaccine can be either RNA or DNA, can have components from prokaryotic as well as eukaryotic sources, and can have the ability, by methods described herein, to enter either eukaryotic or prokaryotic cells and direct expression of isolated nucleic acid molecules of the present

invention in those cells. In a preferred embodiment, a genetic vaccine of the present invention includes a recombinant virus genome (i.e., a nucleic acid molecule of the present invention ligated to at least one viral genome in which transcription of the nucleic acid molecule is directed either by a transcription control region on the genome  
5 or a separate transcription control region) or a recombinant plasmid that includes a nucleic acid molecule of the present invention ligated into a vector that is not a viral genome such that the nucleic acid molecule is operatively linked to a transcription control region.

A genetic vaccine of the present invention includes a nucleic acid molecule of the  
10 present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. Preferred genetic vaccines include at least a portion of a viral genome (i.e., a viral  
15 vector) and a nucleic acid molecule of the present invention. Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, adeno-associated viruses, herpesviruses, picornaviruses, and retroviruses, with those based on alphaviruses (e.g., Sindbis virus or Semliki forest virus), picornaviruses (e.g., poliovirus or mengovirus), species-specific herpesviruses and poxviruses being particularly preferred. Any suitable  
20 transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are  
25 used. The incorporation of a "strong" polyadenylation signal is also preferred.

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intraocular, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600  $\mu$ g, depending on the route of  
30 administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, by gene gun, as drops,

as inhaled aerosols, ingested in microparticles or microcapsules, and/or topical delivery. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (e.g., Sindbis virus), picornaviruses (e.g., poliovirus, mengovirus), raccoon poxviruses, species-specific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published August 18, 1994, which is incorporated by reference herein in its entirety.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of preventing a cat from shedding oocysts as disclosed herein. For example, a recombinant virus vaccine comprising a nucleic acid molecule encoding an immunogenic *T. gondii* protein of the present invention is administered according to a protocol that results in the subject cat producing a sufficient immune response to inhibit shedding *T. gondii* oocysts. A preferred single dose of a recombinant virus vaccine of the present invention is from about  $1 \times 10^4$  to about  $1 \times 10^8$  virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intraocular, intranasal and oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including *Saccharomyces cerevisiae* and *Pichia*

*pastoris*), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about  $10^8$  to about  $10^{12}$  cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

The efficacy of a composition of the present invention to inhibit oocyst shedding caused by *T. gondii* can be tested in a variety of ways including, but not limited to, detection of protective antibodies (using, for example, proteins or mimetopes of the present invention), detection of cellular immunity within the treated animal, or challenge of the treated animal with *T. gondii* to determine whether the treated animal is resistant to oocyst shedding. Challenge studies can include direct administration of *T. gondii* tachyzoites or tissue cysts or sporulated oocysts (the infective stages) to the treated animal. In one embodiment, compositions of the present invention can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

One preferred embodiment of the present invention is the use of immunogenic *T. gondii* proteins, nucleic acid molecules encoding immunogenic *T. gondii* proteins, antibodies and inhibitors of the present invention, to inhibit a cat from shedding oocysts. It is particularly preferred to prevent intestinal stages of the parasite from developing into oocysts. Preferred compositions are those that are able to inhibit at least one step in the portion of the parasite's development cycle that occurs in the intestines prior to the development of oocysts. In cats infected with tissue cysts, for example, the prepatent period for oocyst shedding is three to five days. When cats are infected with sporulated oocysts, for example, the prepatent period can range from 19 to 45 days. Particularly preferred compositions useful for inhibiting oocyst shedding in a cat infected with *T. gondii* include *T. gondii*-based compositions of the present invention. Such compositions include nucleic acid molecules encoding immunogenic *T. gondii* proteins, immunogenic *T. gondii* proteins and mimetopes thereof and anti-*T. gondii* antibodies. Compositions of the present invention are administered to cats in a manner effective to inhibit the cats from shedding *T. gondii* oocysts. Additional protection may be obtained



by administering additional protective compounds, including other *T. gondii* proteins, nucleic acid molecules and antibodies, as disclosed herein.

It is also within the scope of the present invention to use isolated proteins, mimetopes, nucleic acid molecules and antibodies of the present invention as diagnostic reagents to detect infection by *T. gondii*. These diagnostic reagents can further be supplemented with additional compounds that can specifically detect any or all phases of the parasite's life cycle. General methods to use diagnostic reagents in the diagnosis of disease are known to those skilled in the art. A method or a kit for the detection of *T. gondii* infection could be combined with reagents for the detection of additional infectious agents, for example viruses (e.g. Coronaviruses), bacteria (e.g. *Campylobacter*, *Clostridium*, *Salmonella*), protozoa (e.g. *Cryptosporidium*, *Giardia*, *Isospora*, *Hammondia*, *Sarcocystis*, *Besnoitia*, *Microsporidium*), and/or multi-cellular organisms (e.g. *Teania*, *Anclostoma*, *Toxocara*, *Physaloptera*, *Paragonimus*, *Strongyloides*, *Trichuris*).

Another embodiment of the present invention is a method to detect microscopic parasite cysts or oocysts in feces using PCR amplification techniques. By microscopic, it is meant cysts or oocysts that are too small to be conveniently detected by simple visual observation of the feces. Preferred organisms to be detected include oocysts from infectious protozoan parasites including members of the apicomplexa and others including, for example, *Toxoplasma*, *Cryptosporidium*, *Isospora*, *Giardia*, *Eimeria*, *Hammondia*, *Sarcocystis*, *Besnoitia*, *Microsporidium*. Additional infectious agents to detect include, for example, viruses (e.g. Coronaviruses), bacteria (e.g. *Campylobacter*, *Clostridium*, *Salmonella*), and/or multi-cellular organisms (e.g. *Teania*, *Anclostoma*, *Toxocara*, *Physaloptera*, *Paragonimus*, *Strongyloides*, *Trichuris*). Particularly preferred oocysts to be detected include *Toxoplasma* and *Cryptosporidium* oocysts. Preferred cysts to be detected include any cysts capable of binding to a solid support and remaining bound to the support through a washing step. Preferred cysts include *Giardia* cysts. According to this embodiment of the invention, a solid support that is capable of binding cysts or oocysts is contacted with a sample of feces, which may or may not have been partially solubilized first in an aqueous solution, and the sample of feces is allowed

to dry on the support. The solid support can be of any material to which the cysts or oocysts will bind and remain bound during washing in an aqueous solution. The support can comprise one or more compounds that aid in PCR amplification of the sample, for example by allowing the inhibitors to be released in the wash step, or by binding

5 inhibitors of PCR that are not released in the elution step, or by otherwise inactivating inhibitors of PCR amplification. Preferred supports comprise a paper substrate to which the oocysts or cysts can bind. Preferred supports include IsoCodeJ™ Stix, or their equivalent, S&S® #903™, or their equivalent, or Nobuto Blood Filter Strips, or their equivalent. The support, or the portion of the support contacted with the sample of

10 feces, is preferably small enough to fit into a container convenient for the wash step; eg., a size that will fit into a 1.5. ml conical centrifuge tube. The portion of the support that is contacted with the sample of feces can be removed from the rest of the support in order to achieve a convenient size. The portion of the support that includes the dried sample of feces is then washed with an aqueous solution. In a preferred embodiment the

15 aqueous solution is water, preferably distilled water. The solution can comprise one or more compounds that aid in PCR amplification of the sample, for example by inactivating or removing inhibitors of PCR amplification. DNA associated with the sample is eluted by adding an aqueous solution to the support and then heating the solution to a temperature sufficient to elute DNA from the sample, into the solution. In a

20 preferred embodiment, the aqueous solution into which the sample is eluted is water, preferably distilled water. This solution can comprise one or more compounds that aid in PCR amplification of the sample, for example by inactivating inhibitors of PCR amplification, or by improving reaction conditions for the PCR reaction. The heating step comprises heating to a temperature sufficient to elute DNA from the sample. A

25 preferred temperature is approximately 95° C. Oocyst or cyst-specific DNA in the elution solution is then PCR amplified using primers specific to the oocysts or cysts being detected. The amplification products indicative of oocysts or cysts are then detected using any means available for the detection of PCR amplification products. These can include, for example, separation and observation of the PCR products on a

30 gel, or detection and/or quantification by PCR ELISA. In a preferred embodiment of

the present invention, nucleic acid molecules of the present invention are used for the detection of *T. gondii* oocysts in cat feces by PCR amplification using nucleic acid molecules of the present invention as primers. According to the present invention, detection of oocysts can be accomplished by direct analysis of feces. Methods to  
5 conduct such an assay are described further in the Examples section.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

### EXAMPLES

It is to be noted that the examples include a number of molecular biology,  
10 microbiology, immunology and biochemistry techniques considered to be familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.* and Ausubel, et al., 1993, *Current Protocols in Molecular Biology*, Greene/Wiley Interscience, New York, NY, and related references. Ausubel, et al., *ibid.* is incorporated by reference herein in its entirety. DNA sequence analysis and  
15 protein translations were carried out using the DNAsis program (available from Hitachi Software, San Bruno, CA) or MacVector program (available from International Biotechnologies, Inc., New Haven, CT). It should also be noted that since nucleic acid sequencing technology, and in particular the sequencing of PCR products, is not entirely error-free, that the nucleic acid sequences presented herein represent apparent nucleic  
20 acid sequences of the nucleic acid molecules encoding immunogenic *T. gondii* proteins of the present invention.

#### Example 1:

This example discloses the construction of a *T. gondii* genomic expression library.

25 Pure mRNA from *T. gondii* parasite present in the infected cat gut cannot presently be obtained. Therefore, a true cDNA library for the gametogenic stages cannot be produced. In order to get around the unavailability of pure mRNA from gut stages of *T. gondii*, a genomic expression library in  $\lambda$  gt11 was constructed using Toxoplasma genomic DNA obtained from tachyzoites produced in tissue culture. This library  
30 represented genes expressed at all stages of the Toxoplasma life cycle, including the gametogenic genes.

Construction of the library was modeled on procedures used previously for standard lambda cloning (see, for example, Sambrook, et al., *ibid.*). In brief, a series of high frequency cutting restriction enzymes were used to generate near random fragments of DNA representing the tachyzoite genome. DNA fragments of approximately 500 to 2000 bp were size selected and then inserted in frame with the expressed fusion protein in  $\lambda$  gt11. Construction of this library is described in greater detail below.

Standard Production of Tachyzoites from liquid nitrogen stocks: Liquid nitrogen stocks of *Toxoplasma tachyzoites* (TZ) (1 ml samples at  $2-4 \times 10^6$  TZ/ml) were thawed in a 37°C waterbath. The samples were thawed completely without attaining 37°C.

Room temperature TMM (DMEM + 3% FBS + 0.1 ml 50 mg/ml gentamicin per 100 ml media) was added to the thawed sample according to the following timetable: 0.3 ml added at 0 minutes; 0.6 ml added at 5 minutes; 1.5 ml added at 10 minutes. The samples were maintained at room temperature for 5 minutes longer, then centrifuged for 10 minutes at 2,000 RPM at room temperature. The supernatant was discarded and the pellet resuspended in 12 ml of TMM.

Human foreskin fibroblasts (HSF) cells (ATCC CRL 1637) were infected with the thawed tachyzoites as follows: Passage 15-25 HSF cells were split 1:3 and grown to confluence in a T75 flask with DMEM + 10% FBS (fetal bovine serum, available from Summit Biotechnology, Fort Collins, CO) + 0.1 ml gentamicin per 100 ml media in an incubator at 37°C with 5% CO<sub>2</sub>. HSF cells were infected by replacing the media with the thawed tachyzoites in TMM. Infections were allowed to progress until 30-50% of the cell monolayer was destroyed. The medium in the infected T75 flask was replaced with fresh TMM the day before harvesting tachyzoites for expansion of the culture.

Passage 19-25 HSF cells cultured in roller bottles (850 cm<sup>2</sup>), were split 1:3 and grown to confluence in a roller bottle incubator apparatus under conditions as described above. The medium from a single roller bottle was decanted and replaced with 100 ml of TMM. The cells in this roller bottle were then infected by adding medium from an infected T75 flask (described above). Infection was allowed to progress until 30-50% of the cell monolayer was destroyed. Fresh TMM was replaced in the infected roller bottle the day before using the supernatant to infect new HSF cells. Four new roller bottles with confluent HSF cells were each infected with  $2.5 \times 10^7$  tachyzoites harvested from a

previously infected roller bottle. This cycle of infection of four roller bottles, for the purpose of tachyzoite production, was continued on a weekly basis.

Tachyzoite Purification: Extracellular tachyzoites were collected from tissue culture and concentrated. To collect and concentrate tachyzoites, media from roller  
5 bottles containing extracellular tachyzoites were poured into 50 ml conical tubes and centrifuged at 2,000 RPM for 10 minutes. The resulting pellets were pooled and the volume was brought up to 50 ml using TMM. The tachyzoites were diluted and counted using a haemocytometer, and then purified by either the CF-11 column method or the nucleopore method as follows:

- 10 CF-11 Method of Purifying Tachyzoites: 1.5g of CF-11 (available from Whatman, Inc., Clifton, NJ) was mixed thoroughly in 50 ml of DMEM (no FBS), then added to an econo-column chromatography column (available from Biorad, Hercules, CA) and allowed to settle, forming a flat bed. The stopcock was then opened and the excess DMEM was drained until  $\frac{1}{4}$  inch of media remained above the bed. The column  
15 was washed by gently adding 50 ml of DMEM and then bringing the media level down to 1 inch above the CF-11 bed. The 50 ml of tachyzoites in TMM (prepared as described above) was then added to the column. The stopcock was opened and the tachyzoites were eluted at a rate of 1 drop/second and collected into 50 ml conical tubes on ice. The media was eluted to  $\frac{1}{4}$  inch above the gel bed. Two additional 5 ml elutions were  
20 performed, followed by a 40 ml elution. The 100 ml total eluate was then centrifuged at 2,000 RPM for 10 minutes. The pellets were again pooled by resuspension in 50 ml of DMEM. The tachyzoites were counted and the final number of organisms determined. The tachyzoites were centrifuged at 2,000 rpm for 10 minutes, and the pellet resuspended in 1 ml of Hanks Balanced Salt Solution (HBSS). The tachyzoites were  
25 washed 3 times with 1 ml of HBSS by centrifugation at 5000 rpm for 5 minutes in an Eppendorf centrifuge. The pellets were stored at -70°C until needed.

- Nucleopore Method of Purifying Tachyzoites: 47 mm nucleopore units (available from Corning Costar Corp., Cambridge, MA) with a polycarbonate 3  $\mu$ m capillary pore membrane were assembled according to manufacturer's specifications.  
30 The nucleopore units were then placed on top of an open 50 ml conical tube. Five ml of DMEM was gently forced through the unit using a 30 cc syringe that connects to the top

- of the nucleopore unit. Twenty-five ml of the extracellular tachyzoite preparation collected from tissue culture in DMEM were passed through the unit by gently pushing on the 30 cc syringe. The maximum number of tachyzoites per nucleopore filter did not exceed  $5 \times 10^8$ . Filtration was followed by 2, 5 ml washes of DMEM. The nucleopore-
- 5 purified tachyzoites were then centrifuged at 2,000 RPM for 10 minutes, and the pelleted tachyzoites resuspended in 50 ml of DMEM. The number of tachyzoites was determined by counting in a hemacytometer. Following centrifugation at 2,000 rpm for 10 minutes, the pellet was resuspended in 1 ml HBSS. The tachyzoites were washed 3 times with 1 ml of HBSS by centrifugation at 5,000 rpm for 5 minutes in an Eppendorf centrifuge.
- 10 The pellets were stored at  $-70^{\circ}\text{C}$  until needed.

- Isolation of tachyzoite DNA: DNA from all sources (for example, DNA from *Toxoplasma* or mammalian tissue) was isolated using standard techniques that can be found, for example, in Sambrook et al, *ibid*. In particular,  $2 \times 10^9$  tachyzoites were resuspended in 10 ml of 10 mM Tris, pH 8, 0.1 M EDTA, 0.5% SDS and 20  $\mu\text{g}/\text{ml}$
- 15 pancreatic RNase (available from Sigma Chemical Co., St. Louis, MO). After incubating for 1 hour at  $37^{\circ}\text{C}$ , 1 ml of 5M NaCl and 100  $\mu\text{l}$  of 10 mg/ml proteinase K (available from Boehringer Mannheim Corp., Indianapolis, IN) was added and the solution incubated for 3 hours at  $50^{\circ}\text{C}$ . The solution was then extracted with phenol and the DNA precipitated with EtOH.

- 20 Preparation of Restricted and Size Selected DNA: Six, four-base recognition site restriction enzymes, *Alu* I, *Mbo* I, *Msp* I, *Rsa* I, *Sau*3A I, and *Taq* I, (available from New England Biolabs, Beverly, MA) and one six-nucleotide recognition site restriction enzyme, *Dra* I, were used to cut *T. gondii* genomic DNA to completion. Ten  $\mu\text{g}$  of tachyzoite DNA was digested to completion according to the manufacturer's
- 25 recommended protocols for each enzyme. All seven digests of DNA were combined and electrophoresed on an 0.8% preparative agarose gel. The region of the gel representing double stranded DNA between 500 and 2000 bp was excised and the DNA recovered using a Gene Clean Kit (available from BIO 101 Inc., Vista, CA). The eluted DNA was quantitated using an ethidium bromide sensitivity assay on agarose, using calf thymus
- 30 DNA as a standard. The DNA was then ethanol precipitated.

Addition of Linkers: Four  $\mu$ g of the digested and size selected DNA was then prepared for the addition of linkers by filling in the restriction site overhangs as follows: First, the DNA was resuspended into Klenow buffer, 0.2mM dNTPs, and Klenow fragment (available from Boehringer Mannheim Biochemicals, Indianapolis, IN), and the reaction mix was incubated for 30 minutes at room temperature. The reaction was stopped by incubating the reaction mix at 65°C for 10 minutes. The DNA was then methylated using standard conditions including 0.1mM S-adenosylmethionine and 120 units of *EcoR* I methylase (available from Promega Corp., Madison, WI). Following reprecipitation with ethanol, the DNA pellet was dissolved in water and standard T4 DNA ligase buffer (see, for example, Sambrook, et al., *ibid.*). Three separate *EcoR* I linkers, constructed to allow three different reading frames (available from Stratagene, La Jolla, CA) were added along with T4 DNA ligase (available from Promega, Corp.) and incubated for 16 hours at 15°C. The solution was then diluted directly into *EcoR* I restriction buffer and *EcoR* I enzyme (available from Promega Corp.) and incubated at 37°C for 2 hours. The DNA fragments were separated from the free linkers using a Sephacryl S-400 spin column. The recovered DNA was ethanol precipitated.

Ligation and Packaging of the Restricted DNA: The entire fraction of DNA obtained from the above reaction mixture was ligated into 1  $\mu$ g of *EcoR* I-cut and phosphatase treated  $\lambda$  gt11 arms (available from Stratagene) with T4 DNA ligase at 15°C for 16 hours. The phage was then packaged, titered and amplified using the Gigapack® II Packaging system (available from Stratagene) according to the manufacturer's directions. The resulting library is referred to herein as the Toxoplasma or *T. gondii* genomic expression library or as the  $\lambda$  gt11:Toxoplasma genomic expression library.

Example 2:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by antisera specific for a Toxoplasma intestinal stage: oocysts. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

The final stage of *T. gondii* gametogony is the unsporulated oocyst. Antisera was raised directly against Toxoplasma oocysts. In addition to the antisera reacting with their

respective immunogens, the ability of this antisera to react with *T. gondii* gametogenic stages in intestinal tissue sections from infected animals was assessed. When used in immunofluorescence assays conducted on infected cat gut samples, the anti-oocyst antisera reacted with various parasite structures in the ICG tissue sections, indicating some cross-reactivity with gametogenic stages. This antisera was made as follows.

Production of antibody to a *Toxoplasma* intestinal stage: oocysts: Oocysts from a wild type strain designated Maggie, a recent isolate from a cat with Toxoplasmosis (Veterinary Teaching Hospital, Colorado State University, 1993), were obtained from the feces of cats fed mouse brains from mice previously infected with the Maggie strain. The oocysts were purified by the standard method of repeated sugar flotation (described in Dubey, J.P. and Beattie, C.P., (1988) *Toxoplasmosis of Animals and Man*, CRC Press, Boca Raton, FL). The oocysts ( $3 \times 10^7$ ) were vortexed vigorously in 2 ml of PBS, and then frozen and thawed four times using liquid nitrogen and a 37°C water bath. Each thaw was followed with vigorous vortexing. The suspension was then sonicated for 20 seconds. The protein concentration of the sonicate was determined as described above, and the suspension stored at -70° until used.

The thawed oocyst suspension was mixed with Freund's Complete Adjuvant for the first injection and Freund's Incomplete Adjuvant for three subsequent boosts. The protein concentrations of each injection in the series were 9 ug, 50 ug, 90 ug, and 90 ug respectively, delivered at four week intervals. The single cat #1959 (designated Queen 4) used for production of antibody to unsporulated oocysts had been orally infected with 100 mouse brain-derived C strain tissue cysts one month before the initial protein injection. Serum obtained from this cat (designated herein as Q4-1959) was analyzed for the presence of antibody specific to *T. gondii* oocysts by Western blot and immunohistochemistry on a monthly schedule during the injection period.

Immunoscreening the  $\lambda$  gt11:*Toxoplasma* genomic expression library and isolation of *Toxoplasma*-specific nucleic acid molecules reactive with antisera to oocysts: Antisera Q4-1959 was used to isolate nucleic acid molecules herein designated OC-1, OC-2, OC-13, OC-14, OC-22, OC-23 as follows: *E. coli* Y1090 was infected with approximately  $5 \times 10^6$  plaque forming units (PFU) of the  $\lambda$  gt11:*Toxoplasma* genomic expression library, and then evenly spread on 20 LB-amp agarose culture



plates. The phage were allowed to grow for about four hours at 37°C. The plates were then overlayed with nitrocellulose filters impregnated with 10 mM isopropyl-B-D-thiogalactoside (IPTG) to induce the expression of the recombinant Toxoplasma protein. The induction proceeded for between 4 hours to overnight and then the filters were marked to establish orientation. The filters were removed and, following several washes in TBST (Tris-buffered saline (TBS) + Tween 20: 20 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Tween-20), and an incubation in blocking solution (TBS + 5% powdered milk), incubated with a 1:40 dilution of antisera Q4-1959 for about 3 hours at room temperature or overnight at 4°C. After 3 to 5 washes with TBST the filters were incubated with a 1:1000 dilution of alkaline phosphatase (AP) -conjugated goat anti-cat IgG (available from Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) at room temperature for two hours. The filters were washed two times with TBST and once with TBS. The color indicator was developed in AP buffer (100 mM Tris pH 9, 100 mM NaCl, 5 mM MgCl) containing 0.7% NBT (nitroblue tetrazolium) and 0.3% BCIP (5-bromo-4-chloro-3-indolyl phosphate).

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO<sub>4</sub>, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque was isolated. Of the approximately 5X10<sup>6</sup> plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera Q4-1959 were plaque purified.

Characterization of Immunogenic *T. gondii* proteins encoded by nucleic acid molecules selected from the *T. gondii* genomic expression library:

The nucleic acid molecules identified as positive for expression of immunogenic *T. gondii* proteins by immunoscreening with antisera Q4-1959 were screened for expression of proteins reactive with intestinal secretions from immune cats. The production of immune intestinal secretions is described in detail in Example 6, below. Prior to being used for screening, pooled intestinal secretions were preabsorbed with *E. coli* lysates as follows. Individual cultures of *E. coli* Y1090 cells and XL-1 blue cells (available from Stratagene) were grown overnight in LB Amp medium at 37°C. The cells were harvested by centrifugation, then resuspended in PBS, pH 7.4. The cell

suspensions were then frozen and thawed 3 times, using a dry ice-acetone bath and a 37°C water bath, then sonicated on ice for 10 minutes. The protein concentrations of the resulting cell lysates were adjusted to approximately 20 mg/ml, then diluted 1:10 in PBS. Fresh nitrocellulose filters (82 mm) were coated with bacterial proteins by immersing them in the diluted *E. coli* lysates at room temperature for 1 hour. The filters were further incubated in a solution of 4% (w/v) powdered milk in PBS, pH 7.4 for 30 minutes. The filters were then washed with PBS three times for 10 minutes each at room temperature. Pooled immune cat intestinal secretions were diluted 1:20 with 4% (w/v) powdered milk in PBS, pH 7.4. The diluted secretions mixture was incubated with the *E. coli* lysate-treated filters at room temperature for 1 hour, at a ratio of 20 ml per six filters. The resulting absorbed immune intestinal secretions were used without further dilution to screen nucleic acid molecules identified as positive by immunoscreening as described below. Essentially the same protocol was followed when characterizing the proteins expressed by nucleic acid molecules isolated by immunoscreening with other antisera(as described below).

Plaque pure phage identified as positive by immunoscreening were diluted in SM buffer to approximately 50 PFU/3µl. 3 µl of each clone was dropped onto an LB/Amp agar plate which was previously overlayed with top agar containing a 1:20 dilution of a fresh culture of *E. coli* Y1090 at mid-log growth. The plates were then incubated at 37°C for 5 hours. IPTG-treated nitrocellulose filters were placed on the top agar and incubated for 5 hours. The filters were marked, washed in TBS buffer, pH 8.0 at room temperature for 15 minutes and then blocked with 4% (w/v) powdered milk in TBS for 30 minutes, at room temperature. The filters were incubated with absorbed intestinal secretions at 4°C overnight. All further manipulations were at room temperature. The filters were washed in TBS buffer for 10 minutes, 3 times. The filters were incubated for 2 hours with a 1:300 dilution of horse radish peroxidase (HRP) -conjugated goat anti-cat IgA polyclonal antibody (available from Bethyl Laboratories Inc.) in TBS buffer. The filters were washed in TBS for 10 minutes, 3 times, then incubated with 4-chloro-1-naphthol substrate. Clones were judged to be either positive or negative by the intensity of the color reaction relative to wild type phage controls. The results of this assay are summarized in Table 2. Of the six nucleic acid molecules expressing proteins

recognized by antisera Q4-1959, only OC-1 expressed a protein that was positive for reactivity to immune cat intestinal secretions.

Table 2

Nucleic Acid Molecules Selected with Cat Sera Specific to Unsporulated Oocysts

SEQ ID NO	ORIGINAL DESIGNATION	DETECTION				EXPRESSION		pDVAC		REACTIVITY	
		ICG	UCG	TZ	BZ	pTrCHIS	$\lambda$ CRO	IN VITRO	IN VIVO	SERUM	IS
70	OC-1	+	+	+	+	-	ND	ND	ND	ND	+
72	OC-2	+	-	2+	+	+	ND	ND	ND	ND	-
74	OC-13	2+	-	+	+	+	ND	+	+	ND	-
76	OC-14	+	-	+	+	-	ND	ND	ND	ND	-
78	OC-22	2+	-	+	2+	+	ND	+	+	ND	-
80	OC-23	2+	-	2+	+	+	ND	ND	ND	ND	-

Table 2 Legend:

"SEQ ID NO" is the nucleic acid sequence designation for the nucleic acid molecule; "Original designation" is the original name for each nucleic acid molecule; "Detection" represents the results of RT-PCR assays to assess cDNA from infected cat gut cells (ICG), uninfected cat gut cells (UCG), tachyzoites (TZ), and bradyzoites (BZ); "Expression" refers to results of subcloning the nucleic acid molecule into one or both of two *E. coli* expression plasmids, pTrCHIS and  $\lambda$ CRO; "pDVAC" refers to subcloning into and expression from the eukaryotic expression vector pDVAC1, as tested *in vitro* (BHK cells) and *in vivo* (mice); "Reactivity", indicates specific recognition by cat immune sera (serum) and/or cat immune intestinal secretion (IS) of the expressed product. In all cases, (+) indicates a positive response, (-) a negative response, and (ND) indicates not done. In the column labeled "Detection", the numbers associated with the positive responses indicate the relative signal strength for each primer assayed with each of the four cDNA samples, i.e., ICG, UCG, TZ, and BZ cDNA, and are not a comparison between primers.

Some of the nucleic acid molecules identified as positive by immunoscreening were also assessed for expression of proteins reactive with Mozart II (immune) sera. Reactivity was assessed by spotting the purified phage directly on a lawn of host *E. coli* and inducing the expression of protein encoded by the cloned DNA insert using IPTG-soaked filters, similar to the phage screening protocol. The filters were then probed with the Mozart II sera, in essentially the same manner as was used to select the plaque purified phage identified as positive by immunoscreening. The results of these assays are summarized in Table 2.

The Toxoplasma inserts in  $\lambda$  gt11, herein referred to as  $\lambda$  gt11:Toxoplasma nucleic acid molecules were sequenced either by direct sequencing, or by first subcloning the  $\lambda$  gt11:Toxoplasma nucleic acid molecules into a cloning vector, then sequencing. Direct sequencing of each insert was performed as follows: the Toxoplasma-specific insert in  $\lambda$  gt11 was PCR amplified under standard conditions well known in the art using a  $\lambda$  gt11 forward primer (5' GGTGGCGACGACTCCTGGAG 3') and a  $\lambda$  gt11 reverse primer (5' CCAGACCAACTGGTAATGGTAG 3'), and the major PCR reaction product was separated from the rest of the PCR reaction products on a 1% agarose gel. The band representing the major PCR product was excised, and the gel slice was processed using the QIAquick kit (available from Qiagen Inc., Santa Clarita, CA) according to manufacturer's instructions in order to release the DNA. The isolated DNA fragment was sequenced under standard conditions using an ABI PRISM 377 automated DNA sequencer (available from Applied Biosystems, Foster City, CA). Each of the amplification primers were used separately as sequencing primers to obtain sequence from both directions.

Subcloning, then sequencing, was performed as follows: the Toxoplasma-specific insert was PCR amplified and gel purified as described above. The purified DNA was then cloned into a TA cloning vector (available from Invitrogen Corp., San Diego, CA) according to the manufacturer's instructions, and sequenced under standard conditions.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by antisera Q4-1959:

The nucleic acid molecules selected for expression of proteins recognized by antisera Q4-1959 were sequenced as described above. BLASTn and BLASTp homology  
5 searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule OC-1 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as  
10 OC-1-a.

-57-

Table 3

## Homologies

SEQ ID	Size		#	P (N) < 1e-10			TOP HITS		HOMOLOGIES			Size	Clone/Match	Identities	%
	bp	aa		n vs nr	n vs est	p vs nr	Score	Gene	Name						
19	718	99	-	-	-										
21	441	147	-	-	-										
23	428	142	-	-	-										
26	304	101	-	-	-										
28	284	95	-	-	-										
30	690	230	-	-	-										
32	313	54	-	-	-										
34	389	65	-	-	-										
36	548	183	-	-	-										
82	604	112	-	2	-	1.20E-112	AA531653	TgESTzz29d08.r1	invivo Bradyzoite cDNA	553	302-2/8-308	291--301	96		
											446-345/8-109	84--102	82		
											590-489/8-109	82--102	80		
											349-135/129/342	122--214	57		
											493-418/129-204	51--76	67		
											137-16/5-126	69--122	56		
						3.10E-33	AA520213	TgESTzz43d05.s1	TgME49 invivo Bradyzoite	574	363-127/192-428	162--237	68		
											500-364/340-476	117--137	85		
											356-220/340-476	113--137	82		
											601-515/383-469	77/87	88		
											178-106/350-422	51--73	69		
											241-205/456-492	26--37	70		
											373-349/468-492	20--25	80		
84	549		-	2	-	4.30E-35	520213	TgESTzz43d05.s1	TgME49 invivo Bradyzoite	574	113-249/340-476	121--137	88		
											257-403/340-486	123--147	83		

-58-

Table 3

## Homologies

SEQ ID	Size	#	P (N) < 1e-10	TOP HITS	HOMOLOGIES			
							2-105/373-476	93--104 89
							409-530/348-469	84--122 68
							96-120/468-492	20/25 80
					TgESTzz29d08.r1 TgME49 invivo Bradyzoite	553	23-124/8-109	87--102 85
				1.50E-30 AA531653			167-268/8-109	86/102 84
							311-4128-109	77--102 75
							120-195/129-204	54--76 71
85	270 90	-	-					
87	305 102	-	-					
89	804 268	-	2	6.80E-150 N82167	TgESTzy44c02.r1 TgRH tachyzoite cDNA	613	247-498/2-253	245-252 97
							498-557/255-336	79--82 96
							575-620/334-379	44--46 95
				8.00E-40 AA012353	TgESTzz17b0.r1 TgME49 tachyzoite cDNA	380	671-780/1-100	99--100 99
							769-804/98-133	35--36 97
91	867 289	-	1	1.00E-113 N81503	TgESTzy57e07.r1 TgRH tachyzoite cDNA	343	329-541/97-309	211--213 99
							3-151/161-309	147--149 98
93	1424 164	-	2	2.40E-142 AA531653	TgESTzz29d08.r1 TgME49 invivo bradyzoite	553	882-1086/8-212	198--205 96
							1078-1262/206-390	176--185 95
							452-553/8-109	93--102 91
							24-125/8-109	87--102 85
				1.20E-33 AA520213	TgESTzz43d.s1 TgME49 invivo bradyzoite	574	114-250/340-476	119--137 86
							684-820/340-476	117--0137 85
							849-1084/220-455	161--236 68
95	680 227	-	2	3.80E-149 AA520213	TgESTzz43d05.s1 TgME49 invivo Bradyzoite	574	3-352/127-476	343--350 98
							237-493/220-476	202--257 78
				1.50E-37 AA531653	TgESTzz29d08.r1 TgME49 invivo bradyzoite	553	267-501/5-239	168--235 71
							411-512/8-109	86--102 84



-59-

Table 3

## Homologies

SEQ ID	Size	#	P (N) < 1e-10	TOP HITS	HOMOLOGIES			
97	296	99	-	-				
99	723	53	-	-				
101	270	90	-	4.50E-57	AA531653	TgESTzz29d08.r1 TgME49 invivo bradyzoite	553	3-157/236-390
								149--155
								77--108
63	417	139	-	-				71
65	416	138	-	-				
67	500		-	-				
68	321	73	-	-				
54	1233		-	4.50E-176	AA520348	TgESTzz69d04.r1 TgME49 invivo bradyzoites	607	2-216/147-361
								162--215
								239--247
								156--170
55	411	60	-	-				
57	441	118	-	-				
59	491	34	-	-				
61	387	129	-	-				
38	310	95	-	-				
40	220	73	-	-				
42	642	34	-	6.20E-190	AA519977	TgESTzz36d07.r1 TgME49 invivo bradyzoite	653	385-150/199-434
								221--236
								155--164
								124--148
				9.50E-162	AA520558			
				8.90E-122	AA531849			
				1.30E-117	AA520976			
				4.90E-106	AA274332			
				5.20E-102	W99585			
				8.10E-94	AA520425			
				8.40E-90	AA274257			

-60-

Table 3

## Homologies

SEQ ID	Size	#	P (N) < 1e-10	TOP HITS	HOMOLOGIES				
				1.70E-87	AA532000				
				5.00E-81	AA520339				
				2.10E-55	AA012063				
44	381	27	-	9	4.70E-123	AA532000	577	328-3/11-336	316--326
					5.50E-116	AA520339			96
					1.60E-112	AA531849			
					1.70E-100	AA520425			
					3.20E-95	AA519977			
					1.60E-83	AA520558			
					6.80E-59	AA012063			
					4.00E-13	AA274257			
					7.40E-10	W99585			
46	432	85	-	9	4.30E-124	AA520558	441	207-430/91-314	224--224
								119-210/2-93	91--92
					8.10E-120	AA532000			98
					8.90E-113	AA520339			
					2.20E-110	AA531849			
					2.40E-97	AA520425			
					9.90E-94	AA519977			
					8.20E-53	AA012063			
					8.20E-14	AA274257			
					1.50E-10	W99585			
48	282	35	-	-					
50	466	71	-	9	1.70E-125	AA520558	441	119-418/2-316	314--315
					1.30E-116	AA532000			99
					7.70E-110	AA520339			
					4.70E-106	AA531849			

-61-

Table 3

## Homologies

SEQ ID	Size	#	P (N) < 1e-10	TOP HITS	HOMOLOGIES			
				2.60E-97	AA520425			
				2.90E-95	AA519977			
				1.60E-55	AA012063			
				6.40E-14	AA274257			
				1.20E-10	W99585			
52	539	20	8	9.50E-130	AA532000	TgESTzz46d07.r1 TgME49 invivo bradyzoites	577	191-400/85-294
								208-210
								99
								108-190/1-83
								80-83
								96
								397-443/290-336
								46-47
								97
				9.00E-124	AA531849			
				2.50E-109	AA520339			
				2.90E-98	AA520425			
				7.70E-86	AA519977			
				8.30E-83	AA520558			
				4.40E-55	AA012063			
				6.30E-11	W99585			
109	699	233	-	2.70E-40	P46531	Notch protein homolog Homo sapiens	2444	36-72/658-694
			100					19-37
								51
								42-71/243-272
								18-30
								60
								15-40
								37
				3.60E-40	A40043			
				1.60E-35	A36666			
111	419	140	1	1.30E-28	P27951	IGA FC/beta anigen Streptococcus agalactiae	1164	22-139/827-944
			6					40-118
								33
								6-128/823-945
								41-123
								33
				3.40E-28	FCOAG			
				6.20E-22	A60234			
113	303	101	-					
115	696	232	-					
117	173	58	-					

-62-

Table 3

## Homologies

SEQ ID	Size	#	P (N) < 1e-10	TOP HITS	HOMOLOGIES				
119	369 123	-	-						
121	566 61	1	-	2.80E-13 X60241	T. gondii mitochondria-like REP2	1105	459-542/937-1020	69-84	82
			1	2.90E-13 N61888	TgESTzy31c05.r1 TgRH tachyzoite	253	542-460/167-249	68-83	81
123	616 205	-	-						
125	762 254	-	2	5.30E-12 d1017785	hypothetical protein: PE...	1749	5-96/1137-1228	32-92	34
				7.10E-12 S14959					
127	236 79	-	-						
129	569 190	-	-						
131	232	-	-						
132	276 92	-	-						
134	309 103	-	-						
136	534 178	-	-						
139	327 109	-	-						
141	444 148	-	-						
143	928 19	-	-						
70	513 171	-	6	3.60E-15 S14959	proline-rich protein Triticum aestivum	378	10-149/192-331	46-140	32
				3.60E-14 d1017785					
				1.40E-13 160171					
				4.10E-13 1372954					
				4.20E-11 S20500					
				2.90E-10 Q15428					
72	528 176	-	-						
74	375 125	-	-						
76	543 89	-	2	2.00E-72 N82029	TgESTzy39d03.r1	251	525-384/56-197	139-142	97
							386-331/196-251	53-56	94
							542-524/38-56	18-19	94
				1.40E-49 W00112	TgESTzy77b07.r1	401	542-399/136-279	142-144	98



-64-

Table 3

## Homologies

SEQ ID	Size		#	P (N) < 1e-10	TOP HITS		HOMOLOGIES					
	1478	381			4.60E-128	W96667						
7			-	5	-		TgESTzy98f02.r1 TgME49 tachyzoite	454	864-1126/55-317	251--263	95	
									809-868/1-60	72--72	100	
					4.70E-119	AA037916						
					4.50E-43	N82635						
					1.20E-36	N96576						
					2.20E-36	N82193						

## Table 3 Legend:

- Results of BLASTn and BLASTp search of the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database. The algorithm used was as described in S.F. Altschul, W. Gish, W. Miller, E.W. Myers, and D.J. Lipman, J. Mol. Biol. 215, 403-10 (1990) and the NCBI. From left to right: are the
- 5 sequence identification number (SEQ ID No), the size of the nucleic acid molecule (Size) in either base pairs (bp) or amino acids (aa), the number of hits below the sum probability score of  $10^{-10}$  (# P(N) <  $10^{-10}$ ), and a section of the hits with the highest homology (HOMOLOGIES). The homologies section is sub-divided to include the sum
- 10 probability (Score) of the homology, the gene accession number (Gene), the name or identifier of the gene (Name), the size of the gene either in nucleotides, if it is a match in the BLASTn or amino acids if it is in the BLASTp (Size), the range of either nucleotides or amino acids in which a match was identified in the clone versus the match in the database (Clone/Match), the number of identities compared with the range matched
- 15 (Identities), and the percentage homology of the match (%). A dash (-) indicates the search was done and there were no matches.

RT-PCR analysis of nucleic acid sequences encoding Immunogenic *T. gondii* proteins:

The sequence data obtained as described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer  
5 sequences are listed in Table 4.



-67-

Table 4

## Nucleic Acid Molecules Primer Sequences

SEQ ID NO.	ORIGINAL DESIGNATION	NAME	PRIMER SEQUENCE	BASE PAIR NUMBERS
144				
145	Tg-41 (5')	nTG1	CGCTTCTTGTGTACCTG	1-18
146	Tg-41 (3')	nTG1	GCACCTTGTTCTCTCTTCGCC	317-295
147	Tg-45-2T (5')	nTG2	CGAGGAGACGGTGGGAGC	1-18
148	Tg-45-2T (3')	nTG2	TGCCCAAGATGCCGATCTCTG	289-269
149	Tg-50 (5')	nTG4	TCTCCCCCATCGACGAAAAC	95-114
150	Tg-50 (3')	nTG4	GCTCATTTCTCCGCAATTTGG	456-435
151	Q2-4 (5')	nTG5	AGCTGGCAGAAATACCAAAGCTC	67-90
152	Q2-4 (3')	nTG5	TGTCGGCAATACTGGGCATG	529-510
153	Q2-9 (5')	nTG6	ACTGGAGTGGAAAAGTCTGGTTTTG	37-60
154	Q2-9 (3')	nTG6	GACGCAGAGAAGAAAGAGAGCC	415-393
155	Q2-10 (5')	nTG7	TCCAAAACCTGTCTCGTCTCCCC	165-186
156	Q2-10 (3')	nTG7	TCTGGATACGCCGTTCTTTG	305-284
157	Q2-11 (5')	nTG8	GACATCTACCTGTGAGTGAACCAGG	50-74
158	Q2-11 (3')	nTG8	GTCAAAACCTTGCCAGCATCTC	475-454
159	4499-9 (5')	nTG9	TCCGACTGAATGACTACCTCTTTC	45-28
160	4499-9 (3')	nTG9	TCCGACCAAGTCCTCAGTGAAC	537-516
161	4604-2 (5')	nTG10	TGGGCATTTCTGGAAGAGG	36-55
162	4604-2 (3')	nTG10	GAATCCATCTCGTGCAAACGG	378-358
163	4604-3 (5')	nTG11	CAAGACACAGGGAAACGTTGG	102-122
164	4604-3 (3')	nTG11	GAAAGAATCGCACCTCCTCTCC	424-403
165	4604-5 (5')	nTG13	TTTGAGTCTAACC GCCGTATGTC	20-42
166	4604-5 (3')	nTG13	TCAGACGATTCTCCCATTTGACG	216-194
167	4604-10 (5')	nTG15	TCGACTTGGGTCCGATTGTTAG	43-64
168	4604-10 (3')	nTG15	GATCTTTTGC GTGACTTTGTCTGC	289-266
169	4604-17 (5')	nTG16	GAAGATGCTTGTCTTGTTCGGTTC	19-42
170	4604-17 (3')	nTG16	GAGGGGTTTCCTTCTTTATTGCC	178-156
171	4604-54 (5')	nTG17	TGTTGGACATCCCGAGCATC	23-42
172	4604-54 (3')	nTG17	GGTCCTTGTTTTTCAGGCGG	472-453
173	4604-62 (5')	nTG18	TCGTGCAGACAGTGAAGCAATG	35-56
174	4604-62 (3')	nTG18	TTTTGTCAGCACAGAGTGGCG	201-281
175	4604-63 (5')	nTG19	CGCAAGTGAGTTTTGGCTTTACC	15-37
176	4604-63 (3')	nTG19	CCTGGAAGAGATATGCAGACAC	389-368
177	4604-69 (5')	nTG21	TCACCGTTCGCTCTTCTTTCTC	12-33
178	4604-69 (3')	nTG21	CGACTGAAGCATGGATTGCC	367-348
179	AMX/I-5 (5')	nTG31	ACATATTCCTGAGGAGGAGTTCCC	82-105
180	AMX/I-5 (3')	nTG31	AACACACCTCCGACGACACCAC	447-426
181	AMX/I-6 (5')	nTG32	CTCGGCTTCTCCACATACAAGG	8-29
182	AMX/I-6 (3')	nTG32	GGATCTAGGCATTTGGGTTTCAC	411-389
183	AMX/I-7 (5')	nTG33	ATCGAAGAAGCTGAAGCGGAG	4-24
184	AMX/I-7 (3')	nTG33	GTGCTTGTCTCTGACGAAACCC	193-172
185	AMX/I-9 (5')	nTG34	TATCATTTGTATCCCGTCTGCC	47-68
186	AMX/I-9 (3')	nTG34	TGATGCCTGGATTTGCACAAC	363-343
187	AMX/I-10 (5')	nTG35	CGGATCGCTCTGAGTCTCTTTG	1-22

Table 4

## Nucleic Acid Molecules Primer Sequences

188	AMX/I-10 (3')	nTG35	ATCCTGTGTCTTCTCTTCGACCC	384--362
189	AMI-23 (5')	nTG36	GATCGCTCTGAGTCTCTTTG	88--110
190	AMI-24 (5')	nTG37	ACGTGAGGGAGAAGAAGAGAGTGC	21--44
191	AMI-24 (3')	nTG37	TTCATCGTCGCCTCTGATGTCC	347--326
192	AMI-28 (5')	nTG38	TGTAGACAGCGTTTAGGGAGTGC	21--43
193	AMI-28 (3')	nTG38	GTCCTTGGAAGTGCAGAAGCAG	440--419
194	AMI-47 (5')	nTG40	AAGCGAGGAAAAGGAGGTGTC	95-115
195	AMI-47 (3')	nTG40	CGGGAAGGTTGGTGATGTCTGTG	252--230
196	OC-1 (5')	nTG41	CCCGAAGACTTTGACCTG	34--51
197	OC-1 (3')	nTG41	AGTGGCATAGGAGGCTGG	191--174
198	OC-2 (5')	nTG42	GCACCTTCAATGCCACAGGTATC	90--112
199	OC-2 (3')	nTG42	TCGTGTGCTTCTCGCTTCTCTG	484--463
200	OC-13 (5')	nTG43	CACTGTCGATCAGAAGAAGGCTTAC	84--108
201	OC-13 (3')	nTG43	GCTCCGTGGGCACATTTTGTG	367--348
202	OC-14 (5')	nTG44	CAGTTTACGAGGTACAAGGCAACAG	9--33
203	OC-14 (3')	nTG44	GATTGCGTGGGCAGTGTAGAAG	237--216
204	OC-22 (5')	nTG45	TGTTTGTTCCTCCAGTCAACGAC	89--111
205	OC-22 (3')	nTG45	CGGAAGAGGTTGTTGGACTCCTTC	570--547
206	OC-23 (5')	nTG46	CAACCGAGAGAGAAGAGAGGAACAG	62--86
207	OC-23 (3')	nTG46	TGGGGAGAACAGCAGACATCAG	602--581
208	4CQA11 (5')	nTG49	GGATGAACACTGGTGCATCATG	6--27
209	4CQA11 (3')	nTG49	CGACTTGGTCCGCTC	270--256
210	4CQA19 (5')	nTG50	CGGCGGCAACAAATGGGC	1--18
211	4CQA19 (3')	nTG50	GTCCGAGATATGAGGATGCGAC	129--108
212	4CQA21 (5')	nTG51	TCAGAGCACCATTGTTGCGAC	39--59
213	4CQA21 (3')	nTG51	TTTGACGCTCAAGTGGAGGCTG	556--535
214	4CQA22 (5')	nTG52	GCCTGCAACGCTCGATGGC	615--633
215	4CQA22 (3')	nTG52	CTTCTTGACTACCTTCACGTCTG	810--788
216	4CQA24 (5')	nTG53	AAGGACAAGCCTGGTTTG	283--300
217	4CQA24 (3')	nTG53	TTTGCCCTTCGCACAATC	1130--1113
218	4CQA25 (5')	nTG54	CCAGTTTTGCCAGAGGAAGACC	82--103
219	4CQA25 (3')	nTG54	ATCCGTCAATGCAGGTTTCATC	459--438
220	4CQA26 (5')	nTG55	AGACACCAGAGACAGCAGCAGTC	45--67
221	4CQA26 (3')	nTG55	ACTTCGCCCCGACAATCGCTTTCC	266--244
222	4CQA27 (5')	nTG56	CGATCCTCCCGAGGGACC	1--18
223	4CQA27 (3')	nTG56	GCCTTTACGCATTCAAGTCGTG	174--153
224	4CQA29 (3')	nTG57	TTCAGCGGGTCTTTCCTCAC	129--110
225	R8050-2 (5')	nTG58	CAACGAGAAAGATGGAGCTTCG	34--55
226	R8050-2 (3')	nTG58	AACTTCTTGCACTTGGTCCCG	404--384
227	R8050-5 (5')	nTG60	AAGCGAGGAAAAGGAGGTGTCTC	95--118
228	R8050-5 (3')	nTG60	GGAAGGTTGGTGATGTCTGTG	250--230
229	R8050-6 (5')	nTG61	TCCCCCAGGAATTGTTGAAACAG	8--30
230	R8050-6 (3')	nTG61	ACTACCGACAACGTCTCAGTCCTTC	254--230
231	M2A1 (5')	nTG62	CGTGCGTCTGTGAGGAAAAGTG	2--23
232	M2A1 (3')	nTG62	TTGTTGCTCGTGTTCAGGTGC	341--320

Table 4

## Nucleic Acid Molecules Primer Sequences

233	M2A3 (5')	nTG64	TTGTTCTCGAACCCGCAGAG	74-93
234	M2A3 (3')	nTG64	TGGCAAGAGACCGAATCGTG	235-216
235	M2A4 (5')	nTG65	AAACTTGGCAAAGGGGAACG	49-68
236	M2A4 (3')	nTG65	TGCTGTGGAGAATGATGGCTG	483-463
237	M2A5 (5')	nTG66	TTTCCGACGAAGCTGCC	25-41
238	M2A5 (3')	nTG66	GACTCCAACGAAAGCCTCG	144-126
239	M2A6 (5')	nTG67	GGAAAGGGATAAAGACGCCG	150-169
240	M2A6 (3')	nTG67	AAGCAGAGGAGAGACGAGACGAAG	337-314
241	M2A7 (5')	nTG68	CTGCACCATTTCTCACTTCTTG	57-80
242	M2A7 (3')	nTG68	GCAAAAGCGGACTCGATTCTATTG	192-169
243	M2A11 (5')	nTG69	TGTGGCAGAGCAAAAGGCTC	12-31
244	M2A11 (3')	nTG69	CTGTGGATGCTCCTTTGCGACT	406-385
245	M2A16 (5')	nTG70	CGAGGCACCCGAAGAATTTG	195-214
246	M2A16 (3')	nTG70	CTTCTCAGTTCACTTCCTGCG	759-738
247	M2A18 (5')	nTG71	TCACGCAACGAACAAGTCCTC	42-62
248	M2A18 (3')	nTG71	CCCATTTTGTCTTGGCTTGC	149-130
249	M2A19 (5')	nTG72	AGCGGCAAACCAGTTCGTTG	283-302
250	M2A19 (3')	nTG72	CACCACCTTTTTCGTTGCGG	558-539
251	M2A20 (5')	nTG73	CGGCGACTCAGATGGG	1-16
252	M2A20 (3')	nTG73	GGGGCTGTGTCTTCTCTATTTG	131-109
253	M2A21 (5')	nTG74	AAGCAAACAGGCTCGGAAGC	127-146
254	M2A21 (3')	nTG74	TCATGTTGGAGGCGTCGTT	241-222
255	M2A22 (5')	nTG75	TGTGCAGTGGAGGACAAATGG	50-70
256	M2A22 (3')	nTG75	GAATCAGGGTGTTTAGGGCG	284-264
257	M2A23 (5')	nTG76	ATTCTGTGCAAGCCCAGAG	305-323
258	M2A23 (3')	nTG76	CGACCAAGGGTGTTGACCAT	136-155
259	M2A24 (5')	nTG77	CTAGGCAAAGAAACACCCATGC	226-247
260	M2A24 (3')	nTG77	CGCTGGAACCTCTGACAC	327-310
261	M2A25 (5')	nTG78	ACGAAGGGAGAGATGCGTTTG	59-79
262	M2A25 (3')	nTG78	TGGCTGTTTGGGTTGTCTGG	392-373
263	M2A29 (5')	nTG79	TCACCGCAGAACTTAACCCG	62-81
264	M2A29 (3')	nTG79	CTCGCTTTTCCAGCTTGTCG	249-230

## Table 4 Legend:

- 5      Primer Sequences to Nucleic Acid Molecules. The original name (Original Designation) and the present name (Name) for each nucleic acid molecule are listed in the second and third columns. Separate 5' and 3' primer sequences are listed for the nucleic acid molecules under Primer Sequence. Identification of each primer sequence as 5' or 3' is shown in the column labeled Original Designation. The location of each primer sequences in its respective nucleic acid molecule is shown in the column. Base Pair Numbers. The sequence identification number for each primer is listed in the first column (Seq ID NO).

The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. DNA templates were generated from total or poly A+ RNA using an RT-PCT kit (available from Stratagene) according to the manufacturer's instructions. The resulting DNA templates were then amplified by standard PCR reaction. The RT-PCR reactions were performed using RNA isolated from infected cat gut (ICG), bradyzoites (BZ), tachyzoites (TZ), and the appropriate controls (e.g., uninfected cat gut (UCG) RNA). In addition to UCG controls, clone-specific primers were used in PCR reactions using DNA from the following sources: *T. gondii*, mouse cells, cat intestinal cells, and human cells. These results are summarized in Table 2.

Subcloning *T. gondii* nucleic acid molecules encoding Immunogenic *T. gondii* proteins into the expression vector pTrcHisB:

*T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins isolated as described above were subcloned into the expression vector pTrcHisB (available from Invitrogen Corp., San Diego, CA). The vector pTrcHisB is designed for expression of fusion proteins in *E. coli* and purification of proteins encoded by nucleic acid molecules of interest. Expression of fusion proteins from this vector was assessed following induction and subsequent Western blot analysis of the *E. coli* lysates using both a monoclonal antibody to the T7 phage amino acid tag sequence and the original sera used to select the nucleic acid molecule. The fusion proteins all contain a poly histidine amino acid sequence which was used to purify the fusion proteins using metal chelate chromatography.

Recombinant molecules containing nucleic acid sequences encoding immunogenic *T. gondii* proteins were produced by PCR amplifying plaque purified  $\lambda$  gt11:Toxoplasma nucleic acid molecules using a  $\lambda$  gt11 forward primer (5' GGTGGCGACGACTCCTGGAG 3') and a  $\lambda$  gt11 reverse primer (5' CCAGACCAACTGGTAATGGTAG 3'). Amplifying the Toxoplasma inserts in this way produced DNA fragments with *EcoR* I sites at the junctions between the Toxoplasma insert and the lambda vector. These PCR fragments were then digested with the restriction endonuclease *EcoR* I, gel purified and subcloned into the *EcoR* I-

cleaved expression vector, pTrcHisB. The resultant recombinant molecules were transformed into DH5a competent cells to form recombinant cells, and assayed for the expression of an immunogenic *T. gondii* protein. The results of these assays are summarized in Table 2.

5           The recombinant cells were cultured in enriched bacterial growth medium containing 0.1 mg/ml ampicillin and 0.1% glucose at about 37° C. When the cells reached an OD<sub>600</sub> of about 0.4-0.5, expression of recombinant proteins was induced by the addition of 0.5 mM isopropyl-B-D-thiogalactoside (IPTG), and the cells were cultured for about 4 hours at about 37° C. Immunoblot analysis of the recombinant cell  
10   lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. In addition, the original selecting antisera were used to determine whether the recombinant expression molecule expressed a protein that could be recognized by the sera originally  
15   used to isolate the Toxoplasma-specific portion of the recombinant molecule. The results of these immunoblot assays are summarized in Table 2. Of the six nucleic acid molecules selected by immunoscreening with antiserum raised against oocysts (Q4-1959 serum), six were positive by this immunoblot assay.

Example 3:

20           This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by antisera raised against the initiating stage of *T. gondii* gametogony: the bradyzoite. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Antibody To Bradyzoites: Purified C strain bradyzoites ( $3 \times 10^7$ ) from mouse  
25   brain tissue cysts were used to generate stage-specific antibody to *T. gondii* as follows:

*T. gondii* C-strain tissue cysts containing bradyzoites were passaged in mice by harvesting tissue cysts from chronically infected mice that had been infected, either intraperitoneally with tachyzoites produced *in vitro*, or by oral gavage with tissues cysts. Between four and eight weeks post-infection, tissue cysts were harvested and used to  
30   inoculate naive mice. Harvest was accomplished by dissecting out the brains of infected mice euthanized by inhalation of CO<sub>2</sub>. The brains were added to a tube of 30% Dextran

in HBSS (Hanks Balanced Salt Solution, available from Life Technologies Inc. (Gibco/BRL), Gaithersburg, MD), and placed on ice until further purified. Each tube contained a maximum of 8 brains per 20 ml of 30% Dextran solution. Tissue cysts were purified by homogenizing the brains for 20-30 seconds with a Tissuemizer (available  
5 from Tekmar-Dohrmann, Cincinnati, OH). The homogenized brains were centrifuged for 10 minutes at 3,300 g at 4° C. The supernatant was poured off and the pellet was resuspended in 2.0 ml of HBSS. The pellets from multiple tubes were combined and the tissue cysts were counted using a hemacytometer. To produce a new lot of chronically  
10 infected mice, tissue cysts purified as described above were diluted in HBSS to a concentration of 100 tissue cysts/ml. Mice were inoculated by oral gavage with 100 µl (10 tissue cysts). After six weeks there were approximately 600 tissue cysts per mouse.

Bradyzoites were purified from tissue cysts by pepsin digestion and passage through a CF-11 cellulose column. Pepsin digestion was initiated by adding approximately 1.0 ml of pepsin digestion fluid (0.5% pepsin, 0.17 M NaCl, and 1.16 M  
15 HCl) fluid per 1.0 ml of cyst suspension. The sample was incubated for 10 min in a 37°C waterbath with occasional swirling. After incubation, approximately 0.9 ml of 0.5% sodium carbonate per 1.0 ml of sample was added slowly and with constant gentle mixing. The solution was then centrifuged for 10 minutes at 2,000 rpm. The supernatant was removed and the pellet resuspended in 5.0 ml of Dulbecco's Modified  
20 Eagle's Medium.

1.2 g of CF-11 cellulose was added to 50.0 ml of DMEM, and then poured into a 50 ml chromatography column. The column was equilibrated by allowing most of the DMEM to wash out. The pepsin-digested bradyzoites were diluted with 45 ml of  
25 DMEM and loaded onto the column. The column was allowed to drip slowly and the flow through was collected. The column was washed with another 50 ml of DMEM and the flow through was again collected. The two 50 ml flow through aliquots were centrifuged at 2,000 rpm for 15 min. The supernatant was carefully removed and the bradyzoite pellet was resuspended in 1 ml of sterile PBS buffer. The number of  
bradyzoites obtained was determined by counting an aliquot using a hemacytometer.

30 Bradyzoites prepared as described above were lysed in a PBS, 0.001% Triton X-100 solution by freeze-thawing four times in liquid nitrogen and a 37°C water bath. The

resulting lysate was further treated by sonication for ten, 30 second bursts, while on ice. Following protein determination using a BCA Protein Kit (available from Pierce Biochemicals, Rockford, IL), the bradyzoite lysate was mixed with Freund's Complete and Freund's Incomplete Adjuvants for the first and subsequent (booster) injections  
5 respectively. The first injection of rabbit #2448 contained 46 mg of soluble protein, and the two following boosts contained 6  $\mu$ g of soluble protein each. Injections were given subcutaneously at four week intervals, and serum, designated 2448, was collected every three weeks.

Antiserum 2448 was used to isolate nucleic acid molecules herein designated  
10 BZ1-2, BZ1-3, BZ1-6, BZ2-3, BZ2-5, BZ3-2, BZ4-3 and BZ4-6 as follows: *E. coli* Y1090 was infected with approximately  $2 \times 10^5$  PFU and then evenly spread on 4 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a  
15 1:1000 dilution of AP-conjugated goat anti-rabbit IgG. Of the  $2 \times 10^5$  plaques screened in this manner, 8 nucleic acid molecules capable of expressing proteins recognized by antisera 2448 were plaque purified.

Characterization of Immunogenic *T. gondii* proteins encoded by nucleic acid molecules selected from the *T. gondii* genomic expression library:

20 The nucleic acid molecules identified as positive for expression of Toxoplasma stage-specific antigenic proteins by immunoscreening with antisera 2448 were screened for expression of proteins reactive with intestinal secretions from immune cats, as described above. The results of this assay are summarized in Table 5. None of the 8 nucleic acid molecules expressing proteins recognized by antisera 2448 were positive for  
25 reactivity to immune cat intestinal secretions in this assay.



Table 5

Nucleic Acid Molecules Selected with Rabbit Sera Specific to Bradyzoites

SEQ ID NO	ORIGINAL DESIGNATION	DETECTION				EXPRESSION		pDVAC		REACTIVITY	
		ICG	UCG	TZ	BZ	pTrCHIS	λCRO	IN VITRO	IN VIVO	SERUM	IS
38	BZ1-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
40	BZ1-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
42	BZ1-6	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
44	BZ2-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
46	BZ2-5	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
48	BZ3-2	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
50	BZ4-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	-
52	BZ4-6	ND	ND	ND	ND	ND	ND	ND	ND	ND	-

Table 5 Legend: See Legend for Table 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by antisera 2448:

The nucleic acid molecules selected for expression of proteins recognized by antisera 2448 were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule BZ1-2 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as BZ2-1-a.

Example 4:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by rabbit antisera raised against infected cat gut. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of rabbit antisera to infected cat gut: A pregnant female cat (designated Queen 2) (available from Liberty Laboratories, Liberty Corners, NJ) was maintained in isolation and allowed to come to term. The kittens (4) were housed with the mother and nursed normally throughout the protocol. At day seven post-partum, one kitten was selected as the control and its intestine harvested as described below. The remaining kittens were infected orally with 5000 mouse brain-derived tissue cysts of the *T. gondii* strain C, by dripping a solution of the tissue cysts in 1 ml of PBS down the back of their throats. The infected kitten intestines were obtained and processed on day 7 post-infection. The Queen 2 was also infected orally at the same time and in a similar fashion using 100 tissue cysts of *T. gondii* C strain.

In order to obtain fresh intestine, the following procedure was used for both the control and infected animals. A kitten was first anesthetized by placing it in an inhalation chamber which was flooded with both isoflurane and oxygen until the animal was anesthetized. The kitten was then euthanized with an intravenous injection of commercial pentobarbital euthanasia solution at the recommended dose (88 mg/kg). The animal was immediately dissected to expose the small intestine. This was removed by

excisions at the anterior junction with the stomach and the posterior junction with the large intestine. The intestine was opened by a single cut from the anterior to the posterior end, exposing the mucosal surface. The gut was then dipped sequentially into three separate washing baths containing cold HBSS (Hanks buffered saline solution) (available from Life Technologies Inc. (Gibco/BRL), Gaithersburg, MD). The intestine was then placed flat on a chilled laminated sterile surface with the mucosal layer up. A single piece of dry nitrocellulose (BA85, available from Schleicher and Schuell Inc., Keene, NH) the length of the intestine, ranging in size from 40 to 70 cm long (this varied with the animal) and 5mm wide, was carefully placed lengthwise on the mucosal surface of the intestine to obtain an impression smear of the villus epithelial cells. After the nitrocellulose strip became wet (approximately 30 seconds after application), the strip was carefully lifted off and allowed to air dry. The orientation of the anterior and posterior ends of the intestine and strip were noted. Forty biopsy samples, approximately 4 mm by 4 mm sections, were then taken from random positions throughout the length of the intestine, and immediately fixed in either methanol or gluteraldehyde, and maintained for further histological analysis. The intestine was then cut into ten equal sections, and each section placed in a separate bag, labeled and quick frozen in a dry ice and acetone bath. The intestinal sections were maintained at -70° until further processing.

Sections of the cat gut which contained *T. gondii* were identified using PCR analysis of the DNA captured by the nitrocellulose lift with primers specific to the *T. gondii*  $\alpha$ -tubulin gene. The presence of *T. gondii* parasite infection was confirmed by histological analysis of the biopsy sections. Portions of *T. gondii*-positive cat gut sections were then prepared as follows for subsequent injections into rabbits to produce antibody directed toward major epitopes from *T. gondii* gametogenic stages. The same methods were also used to produce antibody in cats to infected cat gut preparations, as herein described (in Example 5). A piece of intestine approximately 2 mm by 20 mm was cut from five frozen sections of infected cat gut material. The pieces were maintained at 4°C, laid flat and the mucosal layer carefully scraped from the intestine wall and muscle layers using a razor blade. This material was then minced and placed in 5 ml of sterile PBS containing 1% nystatin, 10  $\mu$ g/ml gentamicin, and 1%

penicillin/streptomycin in a conical centrifuge tube. The mixture was brought through 4 cycles of a freeze-thaw treatment using liquid nitrogen and a 37°C waterbath. The sample was vortexed between each cycle. The sample was placed on ice and then sonicated using a microtip for 20 seconds followed by 20 seconds on ice. This was repeated four times. The suspension was divided among four Eppendorf tubes and centrifuged (Eppendorf 5415C centrifuge, available from Brinkmann Instruments Inc., Westbury, NY) at maximum speed for 30 minutes at 4°C. The supernatant was then put through a 0.22 micron filter and a protein determination performed using the BCA Protein Determination Kit (available from Pierce Chemical Co., Rockford, IL) according to the manufacturer's protocol. The sample was stored as small aliquots at -70°C until used.

Polyclonal antisera against infected cat gut (ICG) antigens (also herein referred to as anti-ICG antiserum, or anti-ICG antibody) were prepared by immunization of New Zealand White rabbits with infected cat gut tissue protein as follows. Six rabbits were injected with the solubilized cat gut material; two rabbits (designated #4603 and #8049) were injected with solubilized material from uninfected cat gut, and 4 rabbits (designated #4604, #4499, #8050, and #8051) were injected with solubilized material from infected cat gut material. For the first injection, 0.5 mg of soluble protein, prepared as described above, was brought to 0.5 ml and mixed with an equal volume of Freund's Complete Adjuvant. This solution was delivered sub-cutaneously (SQ). The second injection, two weeks later, was identical to the first, except Freund's Incomplete Adjuvant was used. A third injection, twelve weeks after the first injection, was similar to prior injections except that the total amount of protein injected was 1.5 mg. The animals were pre-bled prior to the first immunization and were bled at approximately monthly intervals to monitor antibody responses. The blood was allowed to clot at room temperature and serum obtained by centrifugation. The sera were evaluated for the presence of antibody specific to *T. gondii* by both Western blot analysis using tachyzoite lysates and by indirect immunofluorescent antibody assay (section IFA) using histological sections obtained from infected cat intestine.

The rabbit antisera were preabsorbed to uninfected cat gut material prior to use in immunoscreening, either by absorbing the antisera to Sepharose beads to which

solubilized uninfected cat gut material had been covalently linked, or by absorbing the antisera to nitrocellulose sheets to which uninfected cat gut protein was bound. Western analysis demonstrated that greater than 98% of the serum reactivity to uninfected cat gut was removed by preabsorption to the column. The remaining (unabsorbed) sera showed  
5 reactivity towards *T. gondii* tachyzoite lysates. The unabsorbed sera were used to screen the Toxoplasma genomic library.

Antisera 4604 was used to isolate nucleic acid molecules herein designated 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, 4604-62, 4604-63 and 4604-69 as follows: Two separate immunoscreens were performed with this antisera, and  
10 Toxoplasma-specific nucleic acid molecules were isolated from each screen. In the first screen, *E. coli* Y1090 was infected with approximately  $5 \times 10^4$  PFU and then evenly spread on 10 LB-amp agarose culture plates. In the second screen, *E. coli* Y1090 was infected with approximately  $1.5 \times 10^6$  PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for  
15 immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:500 dilution, and the secondary antibody was a 1:500 dilution of AP-conjugated goat anti-rabbit IgG. Of the approximately  $1.5 \times 10^6$  plaques screened in this manner, 15 nucleic acid molecules capable of expressing proteins recognized by antisera 4604 were plaque purified.

20 Antisera 4499 was used to isolate nucleic acid molecule 4499-9 as follows: *E. coli* Y1090 was infected with approximately  $5 \times 10^4$  PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a  
25 1:500 dilution of AP-conjugated goat anti-rabbit IgG. Of the  $5 \times 10^4$  plaques screened in this manner, 2 nucleic acid molecules capable of expressing proteins recognized by antisera 4499 were plaque purified.

Antisera R8050 (rabbit antisera raised against infected cat gut) was used to isolate nucleic acid molecules herein designated R8050-2, R8050-5, and R8050-6 as  
30 follows: *E. coli* Y1090 was infected with approximately  $5 \times 10^6$  PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as

described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-rabbit IgG (available from Kirkegaard Perry Laboratories). Of the  $5 \times 10^6$  plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by antisera R8050 were plaque purified.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by rabbit anti-ICG antisera were subcloned and sequenced as described in Example 2. The results of assays to characterize the isolated nucleic acid molecules are summarized in Table 6.

Table 6

Nucleic Acid Molecules Selected with Rabbit Sera Specific to Infected Cat Gut

SEQ ID NO	ORIGINAL DESIGNATION	DETECTION				EXPRESSION		pDVAC		REACTIVITY	
		ICG	UCG	TZ	BZ	pTrCHIS	λCRO	IN VITRO	IN VIVO	SERUM	IS
19	4499-9	+	-	+	+	ND	+	ND	ND	+	+
21	4604-2	+	-	+	+	ND	+	ND	ND	ND	-
23	4604-3	+	-	+	-	ND	+	ND	ND	ND	-
25	4604-5	-	-	+	+	ND	-	ND	ND	ND	-
26	4604-10	-	-	+	+	ND	-	ND	ND	ND	-
28	4604-17	+	-	+	+	ND	-	ND	ND	ND	-
30	4604-54	+	-	-	2+	ND	+	ND	ND	ND	-
32	4604-62	+	-	+	+	+	ND	ND	ND	ND	-
34	4604-63	+	-	+	+	-	ND	ND	ND	ND	-
36	4604-69	+	-	2+	+	ND	+	ND	ND	ND	-
103	R8050-2	+	-	2+	+	+	ND	ND	ND	ND	-
105	R8050-5	-	-	+	-	+	ND	ND	ND	ND	-
107	R8050-6	+	-	2+	-	-	ND	ND	ND	ND	-

Table 6 Legend: See Legend for Table 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by rabbit anti-ICG antisera 4604, 4499 and R8050:

Nucleic acid molecules 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, 4604-62, 4604-6, 4604-69, R8050-2, R8050-5, and R8050-6 were sequenced as described above. These nucleic acid molecules were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3., as described above. The results of these searches are summarized in Table 3.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 6.

*T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins isolated by immunoscreening with rabbit anti-ICG antiserum were subcloned into either or both of two expression vectors: pTrcHisB (as described above) or Prcro/T2ori/RSET-B (described below). Expression of the fusion proteins from these vectors, and purification of their expressed fusion proteins, were as described above. The results of assays for the expression of recombinant immunogenic *T. gondii* proteins from these expression vectors is summarized in Table 6.

Recombinant nucleic acid molecules and protein molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET-B: Recombinant molecules containing *T. gondii* nucleic acid molecules operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment, were produced in the following manner. *T. gondii* DNA fragments in  $\lambda$  gt11 were PCR amplified from nucleic acid molecules herein designated 4499-9, 4604-2, 4604-3, 4604-5, 4604-10, 4604-17, 4604-54, and 4604-69, using the  $\lambda$  gt11 forward and reverse primers herein described. Recombinant molecules



were produced by digesting the PCR product with *EcoR* I, gel purifying the resulting fragment, and subcloning into expression vector PRcro/T2ori/RSET-B (also referred to herein as  $\lambda$  CRO) that had been cleaved with *EcoR* I and gel purified. Expression vector PRcro/T2ori/RSET-B contains the following nucleotide segments: An about 1990-bp

5 *Pvu* II to *Aat* II fragment from pUC19 containing the ampicillin resistance gene and *E. coli* of replication; an about 1000-bp *Pvu* II to *Bgl* II fragment from pRK248cIts (available from American Type Culture Collection, Rockville, MD) containing lambda transcriptional regulatory regions (including the gene encoding  $cI^s$ , the promoter  $p_R$ , and a sequence encoding 22 amino acids of the cro protein); an about 60-bp *Bgl* II to *Xba* I

10 fragment from pGEMEX-1 (available from Promega Corp.) which contains the T7 promoter; an about 166-bp *Xba* I to *EcoR* I fragment from pRSET-B (available from Invitrogen, San Diego CA) which contains sequences encoding the T7-S10 translational enhancer, the His<sub>6</sub> fusion, the 14-amino acid S10 leader fusion, and an enterokinase cleavage site as well as the multiple cloning site; and an about 210-bp *EcoR* I to *Aat* II

15 fragment containing synthetic translational and transcription termination signals including the T<sub>1</sub> translation terminators in all three reading frames, an RNA stabilization sequence from *Bacillus thurengiensis* crystal protein and the T<sub>2</sub> rho-independent transcription terminator from the *trpA* operon. Expression vector PRcro/T2ori/RSET-B contains the following nucleotide segments. An about 1990-bp *Pvu*II to *Aat*II fragment

20 from pUC19 containing the ampicillin resistance gene and *E. coli* of replication; an about 1000-bp *Pvu*II to *Bgl*II fragment from pRK248cIts (available from American Type Culture Collection, Rockville, MD) containing lambda transcriptional regulatory regions (including the gene encoding  $cI^s$ , the promoter  $p_R$ , and a sequence encoding 22 amino acids of the cro protein); an about 60-bp *Bgl*II to *Xba*I fragment from pGEMEX-1

25 (available from Promega Corp., Madison WI) which contains the T7 promoter; an about 166-bp *Xba*I to *Eco*RI fragment from pRSET-B (available from Invitrogen Corp., San Diego CA) which contains sequences encoding the T7-S10 translational enhancer, the His<sub>6</sub> fusion, the 14-amino acid S10 leader fusion, and an enterokinase cleavage site as well as the multiple cloning site; and an about 210-bp *Eco*RI to *Aat*II fragment

30 containing synthetic translational and transcription termination signals including the T<sub>1</sub> translation terminators in all three reading frames, an RNA stabilization sequence from

*Bacillus thurengiensis* crystal protein and the T<sub>2</sub> rho-independent transcription terminator from the *trpA* operon.

The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells, using standard techniques as disclosed in Sambrook et al., *ibid*.

5       The recombinant cells were cultured in shake flasks containing an enriched bacterial growth medium containing 0.1 mg/ml ampicillin and 1% glucose at about 32°C. When the cells reached an OD<sub>600</sub> of about 0.6, expression of the Toxoplasma antigen was induced by quickly adjusting the temperature to 42° C and continuing cultivation of the cells for about 2 hours. Protein production was monitored by SDS PAGE of  
10 recombinant cell lysates, followed by immunoblot analysis using standard techniques as described herein and as known in the art. The results of these assays are summarized in Table 6.

The antisera used to originally isolate each Toxoplasma-specific nucleic acid molecule (i.e., either antiserum 4604, or antiserum 4499) was used to identify  
15 recombinant proteins in *E. coli* extracts as follows. The material in crude extracts from *E. coli* were separated by running 5 µg protein per lane on a 12-well 10% Tris-glycine SDS-PAGE gel at 200 volts for 1 hour, and then transferred to nitrocellulose membranes by standard methods. After transfer, the membranes were blocked in 5% (w/v) dry milk for 1 hr at 37°C. The membranes were then incubated with a 1:200 dilution in Tris  
20 buffered saline of the sera originally used to select the nucleic acid molecule encoding Toxoplasma-specific portion of the fusion protein. After 1 hr incubation at room temperature, the blots were washed, and antibody binding resolved using a secondary antibody bound to a substrate for a color indicator. Using the original selecting antibody, immunoblot analysis of *E. coli* lysates identified fusions proteins at or near the  
25 predicted molecular weight of the recombinant fusion protein. The results of these assays are summarized in Table 6.

Histidine tagged fusion proteins were purified from cell lysates as follows. Cell cultures containing nucleic acid molecules of the present invention inserted into either pTrcHisB or λ CRO were grown to an OD<sub>600</sub> of approximately 0.4 to 0.5. The cultures  
30 were induced with IPTG, and the cells harvested 4 hours later. Ten ml of cell culture was centrifuged at 3000 rpm on a table top centrifuge and the protein isolated according

to the manufacturer's instructions using a Ni-NTA Spin Kit (available from Qiagen Inc.). Protein purification was monitored by SDS PAGE followed by Coomassie Blue staining of the column eluate fractions. Recombinant cells including recombinant molecules 4499-9, 4604-2, 4604-3, 4604-54, and 4604-69 produced proteins that were able to bind  
5 to a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant fusion protein.

Recombinant nucleic acid molecules and protein molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding *T. gondii*  
10 antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, *T. gondii* DNA fragments in  $\lambda$  gt11 were PCR amplified from nucleic acid molecules herein designated 4604-62, 4604-63, R8050-2, R8050-5, and R8050-6, using the  $\lambda$  gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells 4604-62,  
15 4604-63, R8050-2, R8050-5, and R8050-6. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. Of the six nucleic acid molecules selected by immunoscreening with rabbit anti-ICG antiserum  
20 that were subcloned into the expression vector pTrcHisB, 15(4604-62, R8050-2, and R8050-5) were positive by this immunoblot assay. The results of these assays are summarized in Table 6.

Example 5:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules  
25 encoding immunogenic *T. gondii* proteins recognized by cat antisera raised against infected cat gut. This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Preparation of cat antibody against infected cat gut: Preparation of infected cat gut material and production of anti-ICG antisera in cats was performed essentially as  
30 herein described for production of rabbit anti-ICG antiserum. Polyclonal cat antisera against infected cat gut (ICG) antigens (also herein referred to as anti-ICG antiserum or

antisera, or anti-ICG antibody) were prepared by immunization of cats as follows. Three cats were injected with cat gut material. One cat (#AME5) was injected with material from uninfected cat gut material and two cats (#AMI4, #AMX1) were injected with material from infected cat gut preparations. The same injection, boost and bleed  
5 regimen and antigen preparation were used for cats as was used for rabbits, described above. Like the rabbit antisera, the cat antisera were preabsorbed to uninfected cat gut material prior to use in immunoscreening.

Anti-sera AMI was used to isolate nucleic acid molecules herein designated AMI-23, AMI-24, AMI-28, and AMI-47 as follows: *E. coli* Y1090 was infected with  
10 approximately  $5 \times 10^6$  PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the  $5 \times 10^6$  plaques screened in this manner, 6 nucleic acid molecules  
15 capable of expressing proteins recognized by antisera AMI were plaque purified.

Anti-sera AMX/I was used to isolate nucleic acid molecules herein designated AMX/I-5, AMX/I-6, AMX/I-7, AMX/I-9, and AMX/I-10 as follows: *E. coli* Y1090 was infected with approximately  $5 \times 10^6$  PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for  
20 immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat anti-cat IgG. Of the  $5 \times 10^6$  plaques screened in this manner, 6 nucleic acid molecules capable of expressing proteins recognized by antisera AMX/I were plaque purified. The results of this immunoscreen are summarized  
25 in Table 7.

Table 7

Nucleic Acid Molecules Selected by Cat Serum Specific to Infected Cat Gut

SEQ ID NO	ORIGINAL DESIGNATION	DETECTION				EXPRESSION			pDVAC		REACTIVITY	
		ICG	UCG	TZ	BZ	pTrCHIS	λCRO		IN VITRO	IN VIVO	SERUM	IS
54	AMX/I-5	+	-	+	+	+	+		ND	ND	ND	+
55	AMX/I-6	2+	-	2+	+	ND	+		ND	ND	ND	-
57	AMX/I-7	2+	-	-	+	ND	+		ND	ND	ND	-
59	AMX/I-9	2+	-	+	+	+	ND		ND	ND	ND	-
61	AMX/I-10	+	+	-	-		+		ND	ND	ND	-
63	AMI-23	+	+	-	-	ND	ND		ND	ND	ND	-
65	AMI-24	+	-	+	2+	+	ND		ND	ND	ND	-
67	AMI-28	+	-	+	2+	ND	ND		ND	ND	ND	-
68	AMI-47	-	-	+	-	+	ND		ND	ND	ND	-

Table 7 Legend: See Legend for Table 2.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by cat anti-ICG antisera were subcloned and sequenced as described in Example 2.

5     Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by cat anti-ICG antisera AMI and AMX/I:

The nucleic acid molecules isolated using antisera AMI or AMX/I were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described  
10     above. The results of these searches are summarized in Table 3.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse  
15     transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 7.

*T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins isolated by immunoscreening with cat anti-ICG antiserum (antiserum AMI or AMX/I) were subcloned into either or both of two expression vectors: pTrcHisB or  
20     Prcro/T2ori/RSET-B (as described above). Expression of the fusion proteins from these vectors, and purification of their expressed fusion proteins, were as described above.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET-B:

25     Recombinant molecules containing *T. gondii* nucleic acid molecules operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment in the vector Prcro/T2ori/RSET-B, were produced essentially as described above, resulting in the production of recombinant molecules. The resulting recombinant molecules were transformed into *E. coli* to form recombinant  
30     cells using standard techniques as disclosed in Sambrook et al., *ibid*. Assays for the

expression of an immunogenic *T. gondii* fusion protein by these cells were performed as described above, and the results are summarized in Table 7.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector

5 pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, *T. gondii* DNA fragments in  $\lambda$  gt11 were PCR amplified from nucleic acid molecules herein designated AMX/I-5, AMX/I-9, AMI-24 and AMI-47  
10 using the  $\lambda$  gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells AMX/I-5, AMX/I-9, AMI-24 and AMI-47. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was  
15 used to confirm the expression of the fusion proteins and to identify their size. The results of this immunoblot analysis are summarized in Table 7.

Example 6:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat immune sera. This  
20 Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of cat immune sera:

Eight specific-pathogen free (SPF) cats (available from Liberty Laboratories, Liberty Corners, N.J.), ages 8-10 months, were randomly assigned to two groups; Group  
25 1, n = 5 and Group 2, n = 3 (the uninfected control group). Before the initiation of any studies with these animals, serum samples were taken from each and tested for reactivity to solubilized tachyzoites. Each animal was seronegative for *T. gondii* by standard Western and ELISA analysis using solubilized tachyzoites as the antigen. This serum also served as the pre-bleed in subsequent studies. Feces from each animal were  
30 analyzed for the presence of shed *T. gondii* oocysts using flotation by sugar solution centrifugation followed by microscopic examination. Food was removed from both

groups fourteen hours prior to Day 0, and on the day prior to all sample collections. On Day 0 the cats in Group 1 were orally inoculated by syringe at the back of the throat with 1000 mouse brain derived *T. gondii* tissue cysts of the Mozart strain. This strain represents an isolate from a cat which presented with Toxoplasmosis at the Veterinary Teaching Hospital, Colorado State University, in 1992. The Group 2 cats were not infected.

The Group 1 cats were housed in individual stainless steel cages in an infectious disease isolation unit. The feces from each animal were collected every day for the first fourteen days post infection (PI) and weekly thereafter until parasite challenge. The feces were analyzed for the presence of shed *T. gondii* oocysts. Five milliliters of whole blood was collected from each animal by jugular venipuncture on the following days post primary infection: 3, 7, 10, 14, 21, 28, 42, 56, 70, 84, 112, 140, 143, 147, 154, 161, 168, and 182.

On day 140 post primary infection, all Group 1 cats were orally challenged with 1000 mouse brain-derived tissue cysts of the Mozart strain. Fecal samples were collected and monitored for the excretion of oocysts for thirty days post challenge (PC). The cats were then bled as before on days: 3, 7, 14, 21, 28, and 42 post challenge.

In addition to the serum samples collected on the bleed dates, both salivary secretions and intestinal secretions were obtained at weeks 0, 1, 2, 4, 8, 10, 16, 20, 21, 22, 23, 24, and 26. These samples were obtained by first anesthetizing each animal with an injection of thiobarbiturate, then intubating the animals and maintaining them with halothane and oxygen. Approximately 0.1 ml of saliva was collected into an equal volume of 0.1 M EDTA. The intestinal secretions were obtained from the upper portion of the small intestine using an endoscope fitted with medical tubing which allowed suction of intestinal fluid. Intestinal secretions were diluted 1:1 with sterile 0.9% NaCl and centrifuged at 10,000 X g for 5 minutes in an Eppendorf centrifuge. The secretions were stored at -70°C until use. Pooled secretions included equal aliquots from all five immune animals from week 20 through 26 post infection. These pooled secretions were used to test the reactivity of intestinal secretions from immune cats to proteins expressed by nucleic acid molecules of the present invention.



All Group 1 animals shed oocysts in their feces during the primary infection and all seroconverted as assessed by Western blot analysis using tachyzoite lysates as the antigen. None of these animals shed oocysts when challenged, and were therefore considered immune. The sera from the immune animals was pooled, and is referred to  
5 herein as Mozart II antiserum or antisera, or as immune antiserum or antisera.

Mozart II antisera was used to isolate nucleic acid molecules herein designated 4CQA-7, 4CQA-11, 4CQA-19, 4CQA-21, 4CQA-22, 4CQA-24, 4CQA-25, 4CQA-26, 4CQA-27, and 4CQA29 as follows: *E. coli* Y1090 was infected with approximately  $8.3 \times 10^5$  PFU and then evenly spread on 13 LB-amp agarose culture plates. The rest of  
10 the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:80 dilution, and the secondary antibody was a 1:50 dilution of monoclonal mouse anti-cat  $\alpha$  chain (available from Serotec, Oxford, England) and the tertiary antibody was a 1:1000 dilution of AP-conjugated goat anti-mouse IgG (Kirkegaard Perry Laboratories). Of the  
15  $8.3 \times 10^5$  plaques screened in this manner, 13 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Table 8

Nucleic Acid Molecules Selected with Immune Cat Sera in Screens II and III

SEQ ID NO	ORIGINAL DESIGNATION	DETECTION				EXPRESSION		pDVAC		REACTIVITY	
		ICG	UCG	TZ	BZ	pTrCHIS	ICRO	IN VITRO	IN VIVO	SERUM	IS
1	Tg-41	2+	-	+	3+	+	ND	+	+	+	-
3	Tg-45	+	-	2+	+	+	ND	+	+	+	+
5	Tg-50	+	-	+	+	+	ND	ND	ND	+	+
82	4CQA-7	ND	ND	ND	ND	ND	ND	ND	ND	+	-
85	4CQA-11	2+	-	+	2+	-	ND	+	ND	+	+
87	4CQA-19	+	-	+	+	-	ND	ND	ND	+	-
89	4CQA-21	3+	-	3+	+	+	ND	ND	ND	+	-
91	4CQA-22	+	-	3+	2+	-	ND	ND	ND	+	-
93	4CQA-24	+	-	2+	3+	-	ND	ND	ND	+	-
95	4CQA-25	+	-	2+	3+	-	ND	ND	ND	+	-
97	4CQA-26	+	+	+	+	-	ND	ND	ND	+	-
99	4CQA-27	+	-	+	+	+	ND	ND	ND	+	-
101	4CQA-29	+	-	+	2+	-	ND	ND	ND	+	-
109	M2A-1	+	-	+	+	ND	ND	ND	ND	+	+
111	M2A-2	ND	ND	ND	ND	ND	ND	ND	ND	+	-
113	M2A-3	-	-	-	-	ND	ND	ND	ND	+	-
115	M2A-4	+	-	+	+	ND	ND	ND	ND	+	-
117	M2A-5	+	-	ND	ND	ND	ND	ND	ND	+	-
119	M2A-6	-	-	-	-	ND	ND	ND	ND	+	-
121	M2A-7	+	-	+	+	ND	ND	ND	ND	+	-
123	M2A-11	+	-	+	+	ND	ND	ND	ND	+	-
125	M2A-16	+	-	ND	ND	ND	ND	ND	ND	+	-
127	M2A-18	+	-	+	+	ND	ND	ND	ND	+	-

Table 8

Nucleic Acid Molecules Selected with Immune Cat Sera in Screens II and III

129	M2A-19	+	+	+	-	+	+	+	ND	ND	ND	ND	+	-
131	M2A-20	+	-	+	+	-	+	+	ND	ND	ND	ND	+	-
132	M2A-21	-	-	-	-	-	-	-	ND	ND	ND	ND	+	-
134	M2A-22	+	-	+	+	-	+	+	ND	ND	ND	ND	+	-
136	M2A-23	-	-	-	-	-	-	-	ND	ND	ND	ND	+	-
139	M2A-24	-	-	-	-	-	-	-	ND	ND	ND	ND	+	-
141	M2A-25	+	-	+	+	-	+	+	ND	ND	ND	ND	+	-
143	M2A-29	+	-	+	+	-	+	+	ND	ND	ND	ND	+	-

Table 8 Legend: See Legend for Table 2.

In addition to the immunoscreen described above, Mozart II antisera was used in another immunoscreen to isolate nucleic acid molecules herein designated M2A1, M2A2, M2A3, M2A4, M2A5, M2A6, M2A7, M2A11, M2A16, M2A18, M2A19, M2A20, M2A21, M2A22, M2A23, M2A24, M2A25, and M2A29 as follows: *E. coli* Y1090 was infected with approximately  $1 \times 10^6$  PFU and then evenly spread on 10 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:50 dilution, and the secondary antibody was a 1:200 dilution of AP-conjugated goat anti-cat IgA (available from Bethyl Laboratories Inc., Montgomery, Texas). Of the  $1 \times 10^6$  plaques screened in this manner, 18 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Mozart II antisera was also used in yet another immunoscreen to isolate nucleic acid molecules herein designated Tg-41, Tg-45, and Tg-50 as follows: *E. coli* Y1090 was infected with approximately  $1 \times 10^6$  PFU and then evenly spread on 12 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:50 dilution, and the secondary antibody was a 1:200 dilution of AP-conjugated goat anti-cat IgA Fc. Of the  $1 \times 10^6$  plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by Mozart II antisera were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 8.

Selected nucleic acid molecules identified by screening for the expression of proteins recognized by Mozart II (immune) antiserum were subcloned and sequenced as described in Example 2.

Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by Mozart II (immune) antiserum:

The nucleic acid molecules isolated using Mozart II (immune) serum were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr)

nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule M2A3 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as M2A3-a. In addition, nucleic acid molecule M2A18 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as M2A18-a.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 8.

Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB:

Recombinant nucleic acid molecules including sequences encoding *T. gondii* antigenic proteins and sequences from the vector pTrcHisB were produced as described in Example 2. In brief, *T. gondii* DNA fragments in  $\lambda$  gt11 were PCR amplified from nucleic acid molecules herein designated 4CQA-11, 4CQA-19, 4CQA-21, 4CQA-22, 4CQA-24, 4CQA-25, 4CQA-26, 4CQA-27, 4CQA-29, Tg-41, Tg-45, and Tg-50 using the  $\lambda$  gt11 forward and reverse primers herein described. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells. Immunoblot analysis of the recombinant cell lysates using a T7 tag monoclonal antibody (available from Novagen Inc., Madison, WI) directed against the fusion portion of the recombinant Toxoplasma fusion protein was used to confirm the expression of the fusion proteins and to identify their size. The results of this immunoblot analysis are summarized in Table 8.

Example 7:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by cat immune sera enriched for

antibodies to gametogenic stages (herein referred to as absorbed immune sera or serum). This Example further discloses recombinant nucleic acid molecules, proteins and cells of the present invention.

Production of cat immune sera enriched for antibodies to gametogenic stages:

5        Sera from cats which were infected and then subsequently challenged with mouse brain-derived tissue cysts were tested for reactivity to extracts of infected cat gut material by Western blot analysis. Sera from one specific cat, designated Queen 2, demonstrated reactivity to particular ICG sections in which the presence of *T. gondii* had been shown by immunofluorescence assay. Queen 2 was originally infected with 100  
10    mouse brain-derived tissue cysts, did not shed oocysts, and seroconverted to positive for tachyzoite antigens by day 39 post-infection. This sera was highly reactive to the asexual stage, tachyzoites. Therefore, to enhance the utility of this sera as a reagent for detection of gametogenic proteins, this sera was used in conjunction with a western blot of infected cat intestinal cell lysates to obtain a fraction enriched in antibody reactive to  
15    the gametogenic proteins. The enrichment of the Queen 2 sera (also referred to herein as Q2 sera) was performed as follows:

      A 12% SDS-PAGE gel was prepared according to standard methods (Laemmli, 1970, *Nature* 227, 680-685). 1000 µg of solubilized ICG protein, prepared as described above, was loaded on 20 x 20 x 0.1 cm gel and run at 8V/cm for 5 hours. Toxoplasma  
20    tachyzoite (TZ) antigen, prepared from solubilized tachyzoites, was used as a control. Separated proteins were transferred to nitrocellulose according to standard procedures for western blotting. After transfer, the nitrocellulose filter was blocked with 4% (w/v) dry milk powder in PBS (pH 7.5), and incubated with a 1:200 dilution of immune cat (Queen 2) antiserum at room temperature for 5 hours with gentle shaking. The filter was  
25    then washed with PBS (pH 7.5). After washing, a 0.5 cm strip was cut off the end of the filter and incubated with a 1:1000 dilution of alkaline phosphatase labeled goat anti-cat IgG antibody at room temperature for 1 hour. The strip was stained with 5-bromo-4-chloro-3-indolylphosphate p-toluene salt/nitroblue tetrazolium chloride substrates (BCIP/NBT)(available from Gibco/BRL). The areas of the gel that stained with  
30    BCIP/NBT substrates represented ICG protein bands which were recognized by IgG antibodies in immune cat serum.

The regions of interest that were visualized on the BCIP/NBT-stained end strip were cut from the remainder of the filter, and the bound antibody eluted with 0.1 M glycine (pH 2.8), 1 mM EDTA at room temperature for 10 minutes. The antibody in glycine was neutralized with 10 mM Tris (pH 9.0), 0.02% NaN<sub>3</sub> was added, and the  
5 solution was stored at 4° C. The purified antibody was analyzed by Western blot of ICG to monitor successful recovery of the eluted antibody, verifying recovery of antibody that reacted with the appropriate molecular weight region of the ICG western blot. This antibody preparation is referred to herein as absorbed immune serum or sera.

The absorbed immune serum was used to isolate nucleic acid molecules herein  
10 designated Q2-4, Q2-9, Q2-10, and Q2-11 as follows: *E. coli* Y1090 was infected with approximately  $3.2 \times 10^5$  PFU and then evenly spread on 8 LB-amp agarose culture plates. The rest of the screening procedure was as described for immunoscreening with antisera Q4-1959 (Example 2), with the following exceptions: the primary antibody was used at a 1:200 dilution, and the secondary antibody was a 1:1000 dilution of AP-conjugated goat  
15 anti-cat IgG. Of the  $3.2 \times 10^5$  plaques screened in this manner, 4 nucleic acid molecules capable of expressing proteins recognized by absorbed immune serum were plaque purified. The results of assays to characterize these nucleic acid molecules are summarized in Table 9.

Table 9

Nucleic Acid Molecules Selected with Absorbed Immune Sera

SEQ ID NO	ORIGINAL DESIGNATION	DETECTION				EXPRESSION			pDVAC		REACTIVITY	
		ICG	UCG	TZ	BZ	pTrCHIS	$\lambda$ CRO		IN VITRO	IN VIVO	SERUM	IS
9	Q2-4	2+	-	+	2+	ND	+		ND	ND	+	-
13	Q2-9	+	-	+	+	-	-		ND	ND	+	-
15	Q2-10	+	-	+	+	ND	+		ND	ND	+	-
17	Q2-11	-	-	+	+	ND	+		ND	ND	+	-

Table 9 Legend: See Legend for Table 2.



Selected nucleic acid molecules identified by screening for the expression of proteins recognized by absorbed immune serum were subcloned and sequenced as described in Example 2.

5     Sequence analysis of nucleic acid molecules selected for expression of proteins recognized by absorbed immune serum:

The nucleic acid molecules selected for expression of proteins recognized by absorbed immune serum were sequenced as described above. BLASTn and BLASTp homology searches were performed on these sequences using the NCBI GenBank™ non-redundant (nr) nucleotide (n) and amino acid (p) databases, and the dbEST (est) database as described above. The results of these searches are summarized in Table 3. Nucleic acid molecule Q2-9 was sequenced again and some changes found between the first and second sequence. The resequenced nucleic acid molecule is referred to herein as Q2-9-a.

The sequence data described above were used to design unique primers specific to each nucleic acid molecule of the present invention. These primer sequences are listed in Table 4. The unique primers listed in Table 4 were used in reverse transcriptase-polymerase chain reaction (RT-PCR) assays to assess the expression of the particular nucleic acid sequence in ICG, bradyzoites and tachyzoites. The results of these assays are summarized in Table 9.

20     Recombinant nucleic acid molecules, protein molecules and cells including sequences encoding *T. gondii* antigenic proteins and sequences from the vector Prcro/T2ori/RSET-B:

Recombinant molecules containing *T. gondii* nucleic acid molecule operatively linked to lambda phage transcriptional control sequences and to a fusion sequence encoding a poly-histidine segment in the vector Prcro/T2ori/RSET-B, were produced essentially as described above, resulting in the production of recombinant molecule. The resulting recombinant molecules were transformed into *E. coli* to form recombinant cells using standard techniques as disclosed in Sambrook et al., *ibid*. Immunoblot analysis of expression of immunogenic *T. gondii* proteins by these recombinant cells is summarized in Table 9.

Example 8:

This Example describes the construction of several cDNA expression libraries of the present invention.

A *T. gondii* tachyzoite cDNA expression library, a *T. gondii* infected cat gut (ICG) cDNA library (constructed from seven day post infection infected cat gut material, which is a mix of both cat intestinal cDNA and *T. gondii* gametogenic cDNA), and an uninfected cat gut (UCG) cDNA expression library were from total RNAs as follows:

Isolation of Total RNA From Tachyzoites: Total RNA from tachyzoites was prepared using Tri-Reagent™ (available from Molecular Research Center, Inc, Cincinnati, Ohio) according to the manufacturer's directions. Briefly, 4 X 10<sup>9</sup> tachyzoites were resuspended in 6 ml of TriReagent with a syringe and 18 gauge needle. Successive triturations were made with 20 gauge and 22 gauge needles. A volume of CHCl<sub>3</sub> equal to one-fifth the original volume of TriReagent® was added and the mixtures were shaken for 15 seconds. The aqueous and organic phases were then separated by centrifugation. Total RNA was recovered from the aqueous phase by precipitation in isopropanol.

PolyA<sup>+</sup> RNA was isolated from total RNA using Pharmacia mRNA purification kit (available from Pharmacia Biotech Inc., Piscataway, NJ).

Isolation of Total RNA from Other Sources: The method of isolation of total RNA from various tissues was the same for all tissues. The only variable was the starting material. For example, to obtain RNA from infected cat gut (ICG) or uninfected cat gut (UCG), the epithelial layer of a fifteen square centimeter section of gut was scraped into 6 ml of Tri-Reagent and processed as described above. RNA from mouse was obtained from 1 gm of mouse brain and treated with Tri-Reagent as described above. RNA from bradyzoites was obtained from 7,000 tissue cysts propagated in mouse brain, obtained as described, and treated with Tri-Reagent as described above.

PolyA<sup>+</sup> mRNA was isolated from total RNA using Pharmacia mRNA purification kit (available from Pharmacia Biotech Inc., Piscataway, NJ).

Preparation of  $\lambda$  cDNA libraries:

The ZAP-cDNA<sup>®</sup> synthesis kit (available from Stratagene) was used according to manufacturer's instructions to synthesize cDNA. Briefly, 5 or 10  $\mu$ g of PolyA<sup>+</sup> mRNA (prepared as described above) was reverse transcribed using Superscript<sup>®</sup> reverse transcriptase and 0.6 mM dGTP, dATP, dTTP, and 0.3 mM 5-methyl dCTP and 1.4  $\mu$ g of oligo dT linker primer supplied with the ZAP-cDNA<sup>®</sup> Synthesis Kit. The second strand was made by digesting the RNA template with RNaseH and priming second strand synthesis with DNA polymerase I. The cDNA was then ligated into the Uni-ZAP<sup>®</sup> XR lambda insertion vector (available from Stratagene), packaged and amplified to produce tachyzoite and ICG cDNA libraries.

5  $\mu$ g of polyA<sup>+</sup> RNA was used to prepare the ICG cDNA library, and 10  $\mu$ g of polyA<sup>+</sup> RNA was used to prepare the tachyzoite cDNA library. For each library, 100 ng of double stranded cDNA was ligated and packaged and gave approximately  $1.5 \times 10^6$  unique nucleic acid molecules. The average size of the cloned inserts was 1.9 Kb in the tachyzoite cDNA library, and 2.1 Kb in the ICG cDNA library.

Example 9:

This Example describes the construction and identification of cDNA sequences encoding near full-length *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins.

Two of the molecular libraries described above were used to isolate near full-length *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins: the tachyzoite cDNA library and the ICG cDNA library constructed from seven day post infection infected cat gut material.

The general approach to isolating nucleic acid sequences representing full length, or near full-length cDNA sequences was as follows: First, the MacVector DNA analysis program was used to design DNA primers for each of the Toxoplasma sequences cloned in an expression vector as herein described. These primers were then used in a PCR reaction in which the template was either of the Toxoplasma cDNA libraries herein described. The presence of a positive band on an agarose gel following PCR was diagnostic of the presence in the cDNA library of a nucleic acid molecule with homology to the primers. A near full-length cDNA molecule having sequence homology with the

genomic DNA sequence designated Q2-4 was obtained by a direct hybridization screen of the libraries using radiolabeled clone-specific PCR fragments as templates. The isolation of one of these near full-length sequences is herein described in detail as representative of the methods used to isolate all of the near full-length sequences identified by this strategy.

A cDNA sequence representing a near full-length gene having homology to a nucleic acid sequence herein designated Q2-4 (isolated from the *Toxoplasma* genomic DNA library) was isolated from the infected cat gut (ICG) cDNA library by hybridization screening as follows: *E. coli* Y1090 was infected with approximately 1X10<sup>6</sup> PFU of the *Toxoplasma* ICG cDNA library and then plated at a density of about 50,000 plaques per 150 mm agar plate. The resulting plaques were transferred to nitrocellulose filters. The filters were then soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for two minutes, neutralization solution (1.5 M NaCl, 0.5 M Tris, pH 8) for five minutes, and then rinsed several times in 2 X SSC (150 mM NaCl, 15 mM Na citrate, pH 7). The DNA bound to the filters was crosslinked using a Stratalinker<sup>®</sup> UV crosslinker (available from Stratagene) according to the manufacturer's directions.

A radioactive hybridization probe was made by incorporating <sup>32</sup>P into clone-specific template DNA using a Prime-It<sup>®</sup> II random primer labeling kit (available from Stratagene) following the manufacturers directions. The template was a PCR fragment generated by using two primers specific for Q2-4. For each 100 µl reaction, 30 ng of *Toxoplasma* genomic DNA was PCR amplified using 200 mM of each dCTP, dGTP, dTTP, dATP, 200 nM of each specific primer, 2.5 mM MgCl<sub>2</sub>, 20 mM Tris pH 8.4, 50 mM KCl, and 2.5 units *Taq* DNA polymerase (available from The Perkin Elmer Corp.) for thirty-five cycles in a Perkin-Elmer Gene Amp PCR System (available from The Perkin Elmer Corp.).

The nitrocellulose filters containing crosslinked DNA were hybridized in 2 X PIPES buffer (10 mM piperazine-N, N'-bis[2-ethanesulfonic acid] (pH 6.5), 400 mM NaCl), 50% formamide, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA and 10<sup>7</sup> cpm/ml of the radioactive hybridization probe. The filters were incubated with this hybridization solution overnight at 42<sup>o</sup> C. The next day the filters were washed in 0.1 X

SSC, 0.1 % SDS and then exposed to X-ray film (available from Kodak, Rochester, NY) in order to visualize positive plaques.

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO<sub>4</sub>, and 0.01% gelatin) and the  
5 phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque corresponding to a full length cDNA nucleic acid sequence representing Q2-4 was isolated.

After plaque purification, the nucleic acid molecules were mapped and the areas of interest sequenced using primers specific to the original clone, long fragment PCR,  
10 and cycle sequencing of the large fragments.

Example 10:

This Example describes the expression in a eucaryotic cell of nucleic acid molecules encoding immunogenic *T. gondii* proteins, and DNA vaccination with nucleic acid molecules encoding immunogenic *T. gondii* proteins.

15 Cloning into a eucaryotic expression vector(pDVacI):

Inserts from eight clones (OC-2, OC-13, OC-14, OC-22, Tg-41, Tg-45, Tg-50, 4CQA-11) were ligated into the pDVacI expression vector. This vector contained a eucaryotic promoter from cytomegalovirus (CMV), followed by the start codon and signal sequence for a mouse kappa immunoglobulin gene. An *EcoR* I site was inserted in  
20 frame downstream to the signal sequence. This allowed the insertion of *EcoR* I fragments directly from the original lambda phage. The nucleic acid molecules produced by insertion of nucleic acid molecules encoding immunogenic *T. gondii* proteins into pDVacI are referred to herein as pDVacI:Toxoplasma nucleic acid molecules. If the *EcoR* I inserts represent nucleic acid sequence that is entirely open  
25 reading frame, then the protein product expressed from these inserts may be in frame with a C-terminal fusion consisting of both a poly histidine track and amino acid sequence representing an epitope from the human *myc* gene as a reporter sequence. The N-terminal fusion adds 49 amino acids, or about 5.4 kD to the protein encoded by the *T. gondii* nucleic acid molecule, and the C-terminal fusion adds 38 amino acids, or about  
30 4.2 kD, to the fusion protein.

Expression *in vitro*:

Direct sequencing of the inserts in each plasmid confirmed the production of eight different pDVacI:Toxoplasma nucleic acid molecules. DNA from these molecules was then tested for eukaryotic expression of antigenic *T. gondii* proteins by transfecting

5 BHK cells *in vitro* with DNA isolated from the pDVacI:Toxoplasma nucleic acid molecules. Analysis of the eukaryotic expression products of the pDVacI:Toxoplasma nucleic acid molecules was done by western blot on cell lysates and on supernatants from the transformed BHK cells. Either a monoclonal reactive with the *myc* epitope or antibody specific to each clone was used as the primary antibody. Seven out of the eight

10 plasmid constructs expressed a protein *in vitro*. See Table 10.

Table 10  
Analysis of Clones in Eucaryotic Expression Vector and DNA Vaccination

Clone	Size (KD) Expressed in pDVac	EU / ug DNA*	Expression <i>in vitro</i>		Sero- conversion (# of Mice)**
			Pellet	Super	
OC-2	40	0.3 / 0.4	+	+	5 / 5 / 5
OC-13	38	0 / 0.23	+	+	0 / 0 / 4
OC-14	32	7.7 / 3.8	-	-	***
OC-22	40	0.5 / 0.44	+	+	4 / 5 / 5
Tg-41	33	23 / 1.8	+	+	0 / 1 / 5
Tg-45	26	0 / 0	+	+	3 / 5 / 5
Tg-50	55	4.0 / 4.0	+	+	5 / 5 / 5
4cqa-11	25	0.95 / 5.3	+	+	0 / 0 / 0

Table 10 legend:

(\*) The first and second numbers represent the endotoxin units (EU)/ug of DNA for the first and second immunizations respectively. (\*\*) The numbers represent the # of mice that sero-converted at the 4, 7, and 9 week bleeds, respectively, out of the group of five that were injected. (\*\*\*) Antigen for Nt4 protein was not available to analyze for these sera samples.

Expression in vivo:

100 ug of each pDVacI:Toxoplasma nucleic acid molecule was injected intradermally into five mice. The administrations were at day zero and week five; bleeds were collected at weeks four, seven and nine. The mouse sera were used to determine if the DNA vaccination with each clone elicited a serological response to the cloned fusion protein. This was measured by western blot analysis with the protein expressed in the BHK lysates. Six of the eight clones induced antibodies in mice by week nine, see Table 10.

Reactivity of antibody raised against recombinant OC-1 protein:

Purified recombinant protein expressed by an expression vector containing the nucleic acid sequence referred to as OC-1 (SEQ ID NO:70) was used to immunize mice and rabbits by methods well known in the art. The animals were bled, and serum collected used in immunofluorescence assays against infected and uninfected cat gut tissue. The results of these assays showed that antibody raised, in mice and rabbits, to recombinant OC-1 protein bound to most of the enteroepithelial stages in the infected cat gut. The antiserum did not react with uninfected cat gut.

Example 11:

This example describes the construction of a *Toxoplasma gondii* EMBL3 genomic library from tachyzoites grown in tissue culture. This Example further describes isolation of near full-length nucleic acid molecules encoding stage specific *T. gondii* antigenic proteins.

An EMBL3 library of *Toxoplasma* genomic DNA was constructed using standard molecular cloning methods, well known to those skilled in the art of cloning (see, for example, Sambrook, *et al.*, *ibid.*). In brief, *Toxoplasma* genomic DNA, prepared from tachyzoites as herein described, was partially digested with *Sau3A* I, using a series of different ratios of units of enzyme to  $\mu\text{g}$  of DNA. Digestions were incubated at 37°C for one hour. Ratios of 0.06, 0.03, and 0.015 units of enzyme per  $\mu\text{g}$  of DNA produced high molecular weight DNA fragments which were then run on a preparative agarose gel. The fraction of the gel corresponding to DNA in a size range of between 15 and 20 Kb was excised. The DNA fragments were extracted from the gel, and the amount of extracted DNA quantitated. The EMBL3 library was then constructed



using this DNA and the Lambda EMBL3/*Bam*H I Vector Kit (available from Stratagene). The manufacturer's instructions were followed for all cloning steps, and the resulting ligated DNA was packaged using the Gigapack<sup>®</sup> II XL Packaging Extract (available from Stratagene). Packaging and amplification followed the manufacturer's specifications. The resulting library is referred to herein as the EMBL3:Toxoplasma genomic library.

The EMBL3:Toxoplasma genomic library was plated at a density of 50,000 plaques per 150 mM agar plate and the plaques transferred to a nitrocellulose filter. The filters were soaked in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for two minutes, neutralization solution (1.5 M NaCl, 0.5 M Tris, pH 8) for five minutes, rinsed several times in 2 X SSC (150 mM NaCl, 15 mM Na citrate, pH 7), and the DNA crosslinked using a Stratalinker<sup>®</sup> UV crosslinker (available from Stratagene) according to the manufacturer's instructions.

The EMBL3:Toxoplasma genomic library was screened with probes made from PCR amplified nucleic acid molecules isolated by immunoscreening the  $\lambda$  gt11:Toxoplasma genomic library. The primers used to generate these probes were derived using the MacVector Sequence Analysis program and the sequences of nucleic acid molecules encoding *T. gondii* antigenic proteins isolated from the  $\lambda$  gt11:Toxoplasma genomic library.

The filters were hybridized in 2 X PIPES buffer (10 mM piperazine-N, N'-bis[2-ethanesulfonic acid] (pH 6.5), 400 mM NaCl), 50% formamide, 0.5% SDS, 100  $\mu$ g/ml denatured salmon sperm DNA (available from Sigma) and 10<sup>7</sup> cpm/ml of radioactive hybridization probe. The filters were hybridized overnight at 42°C. The next day the filters were washed in 0.1 X SSC, 0.1 % SDS, and then exposed to X-ray film (Kodak).

Plaques in the area corresponding to the positive signals were picked into SM buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 8 mM MgSO<sub>4</sub>, and 0.01% gelatin) and the phage replated at a lower density. The same screening procedure was repeated three or four times until a pure plaque hybridizing with a nucleic acid molecule isolated by immunoscreening the  $\lambda$  gt11:Toxoplasma genomic library was isolated. After plaque purification, the nucleic acid molecules were mapped and the areas of interest sequenced

using primers specific to the original clone, long fragment PCR, and cycle sequencing of the large fragments.

Long fragment PCR was done with a Perkin-Elmer XL PCR kit (available from The Perkin-Elmer Corp., Foster City, CA) as follows: A 100 µl reaction was separated  
5 into two layers with a wax bead so one would have a hot-start reaction. The lower layer contained 1 X XL PCR buffer supplied with the kit, 40 pM each of the forward and reverse primers, SC1011 and SC1002, (supplied by the manufacturer with the XL PCR kit, 2.5 mM each dNTP, 1.1 mM Mg(OAc)<sub>2</sub>. The upper layer contained 1 X XL buffer, 4 units of rTth DNA polymerase (available from The Perkin-Elmer Corp.) and about 5  
10 ng of the plaque purified EMBL3:Toxoplasma genomic DNA as the template. The reaction was done in a Hybaid thermocycler (available from Hybaid Ltd., Middlesex, UK), and the reaction products were resolved on a 0.6% agarose gel.

Example 12:

This Example describes the detection of *T. gondii* oocysts in cat feces by PCR  
15 amplification of nucleic acid sequences homologous to nucleic acid sequences encoding immunogenic *T. gondii* proteins of the present invention. Specifically, this example describes a rapid PCR dipstick method for the detection of oocysts in feces.

Naive cats were infected per os by 1000 mouse-brain derived tissue cysts of *T. gondii* strain C at day zero. Feces from each animal were collected, if available, on a  
20 daily basis starting at day zero and each day for 19 days post infection (PI). A portion of the feces was treated by the standard sugar floatation method (Dubey, J.P., Swan, G. V., and Frenkel, J. K. 1972, Journal of Parasitology. 58: 1005-1006) and the oocysts visualized using a microscope and counted on a haemocytometer. A portion of each feces was also suspended in PBS, vortexed and a small sample obtained by dipping an  
25 IsoCodeJ™ dipstick (available from Schleicher & Schuell, Keene, NH) into the fecal solution. The dipstick was allowed to air dry and then washed in 500 µl of distilled water by vortexing the stick end and water in a tube for 10 seconds. Material adhering to the filter was then eluted in 50 µl of fresh distilled water by heating to 95° C for 30 minutes. The remaining supernatant was then used for standard hot start PCR, according  
30 to methods well known in the art, using primers representing DNA sequences from nucleic acid molecules encoding *T. gondii* antigenic proteins. The results of an

experiment in which primers derived from nucleic acid molecule OC-2 were used are shown in Table 11. The results of this experiment demonstrated that the PCR detection method was at least as sensitive at detecting oocysts in fecal matter as the conventional floatation method.

-110-

Table 11

## PCR Analysis of Cat Feces

#3528-U				#3512-I				#3515-I			
Day	Oocysts/gm	PCR		Day	Oocysts/gm	PCR		Day	Oocysts/gm	PCR	
PI	Float	Dipstick	Oc2	PI	Float	Dipstick	Oc2	PI	Float	Dipstick	Oc2
0	0	-	-	0	0	-	-	0	0	-	-
1	0	-	-	1	0	-	-	1	0	-	-
2	0	-	-	2	0	-	-	2	0	-	-
3	0	-	-	3	0	-	-	3	0	-	-
4	0	-	-	4				4	1X10e6	+	+
5	0	-	-	5	1X10e4	+	+	5	5X10e6	+	+
6	0	-	-	6	3X10e5	+	+	6	1X10e6	+	+
7	0	-	-	7	1X10e6	+	+	7			
8				8	1X10e6	+	+	8	2X10e5	+	+
9	0	-	-	9	1x10e6	+	+	9	7X10e4	+	+
10	0	-	-	10	1X10e5	+	+	10	0	+	+
11	0	-	-	11	1X10e5	+	+	11	0	-	-
12				12	0	+	+	12	0	-	-
13				13	0	-	-	13	0	-	-
14				14	0	-	-	14	0	-	-
15	0	-	-	15	0	-	-	15	0	-	-
16				16				16	0	-	-
17				17				17	0	-	-
18				18	0	-	-	18	0	-	-
19	0	-	-	19	0	-	-	19	0	-	-

A series of additional experiments was performed in order to investigate further the PCR dipstick method for the detection of oocysts in feces. In this set of experiments, the following methods were used to produce *T. gondii* infected cats, and to detect oocysts in the feces of the infected cats. *T. gondii* C-strain tissue cysts were obtained by orally infecting 6-8 week old Swiss Webster mice with a sub-lethal dose of mouse brain derived tissue cysts. At six weeks post infection, the animals were euthanized with CO<sub>2</sub> and the brains were removed and placed in 30% Dextran in HBSS (Gibco/BRL). The brains were then homogenized with a Tissuemizer (Tekmar Co., Cincinnati OH) and centrifuged at 5,000 x g's for 10 min at 4°C. The pellet was resuspended in HBSS and the tissue cysts were counted. The tissue cysts were diluted with PBS to the appropriate concentration for oral administration to cats at the back of the throat using a 1ml syringe. A total of twenty cats was used in this study: seventeen were experimentally infected with 1000 tissue cysts and three were used as uninfected controls. All cats were housed in separate cages and feces were collected at the day of infection and daily for the next 21 days. On average there were approximately twelve samples per cat. The fecal samples were stored at 4°C until tested, which was within two weeks of collection.

Conventional quantification of oocysts in feces was based on the sugar flotation method of Dubey and Beattie, 1988, and is described in full as follows. Each fecal sample was weighed and then 2 grams of feces were mixed with 15 ml of sugar solution (53 gm sugar, 100 ml of water). Following solubilization with a tongue depressor, the mixture was passed through two layers of gauze. The filtrate was poured into a 15 ml conical tube and centrifuged at 1,200 x g for 10 minutes. The top 3 ml of the sample was added to 13 ml of sugar solution and centrifuged as above. The top 3 ml of the second flotation was added to 13 ml of water and centrifuged at 1,200 x g for 10 minutes. The resulting oocyst pellet was resuspended in 1ml of water and the oocysts counted using a hemacytometer. Alternatively, the entire fecal sample was solubilized in PBS by adding five ml of PBS per gram of the pre-weighed feces in a 250 ml plastic beaker. After one hour at room temperature, a tongue depressor was used to thoroughly suspend the feces. Five ml of the fecal slurry was added to a 15 ml tube containing 5 ml of 2X sugar solution and inverted several times. The tube was then centrifuged at 1,200

x g for 10 minutes. The top 3 ml of the sample was subjected to a second sugar flotation, resuspended, and counted as described above.

Analysis of the fecal samples by the PCR dipstick method was performed as follows. One ml aliquots were taken, prior to further processing for floatation, from  
5 each of the initial fecal slurries described above. Samples were collected directly onto dipsticks, either by spotting 10 ul onto each dipstick filter or by directly dipping the dipstick into the fecal slurry. The filters were then dried at room temperature and the filter portion of the dipstick was cut off into a sterile 1.5 ml centrifuge tube. The filter was washed with 500 ul of sterile distilled water by vortexing for 8 seconds. The wash  
10 was removed and 50 ul of sterile water was added to the tube and adherent oocyst DNA eluted by heating at 95°C for 1 hour. The filter was removed with a sterile tip and the sample stored (also referred to as the dipstick eluate) at -20°C.

Primers specific to two *T. gondii* genes, *B1* and OC-2, were used in the amplification reactions. The primers for the *B1* gene (Burg, et al., 1989, *Journal of*  
15 *Clinical Microbiology*, 27: 1787-1792) were *B1* forward (5'-GGA ACT GCA TCC GTT CAT GAG-3', herein referred to as SEQ ID NO:332), *B1* reverse (5'- TCT TAA AGC GTT CGT GGT C-3', herein referred to as SEQ ID NO:333), and a *B1* internal primer (5'-GGC GAC CAA TCT GCG AAT ACA CC-3', herein referred to as SEQ ID NO:334). The *T. gondii* OC-2 was isolated as herein described. The OC-2-derived  
20 primers were OC-2 forward (5'-GCA TCC TTG GAG ACA GAG CTT GAG-3', herein referred to as SEQ ID NO:335), OC-2 reverse (5'-GGG TTC TCT TCT CGC TCA TCT TTC-3', herein referred to as SEQ ID NO:336), and an OC-2 internal primer (5'-AGT CAG AAG CAG TCA AGG C-3' herein referred to as SEQ ID NO:337). The PCR mixture contained 1X PCR buffer (10 mM Tris-HCl<sub>2</sub>, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl), 0.2  
25 mM deoxynucleoside triphosphates (Perkin-Elmer Cetus Corp., Norwalk, CN), 0.8 uM of each primer, 0.5 U of Gold AmpliTaq™ DNA polymerase (Available from Perkin-Elmer Corp.), and 1 ul DNA template in a total volume of 25 ul. The reaction mixture was denatured at 95°C for 10 minutes, amplified for 42 cycles including a denaturation step at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 40  
30 seconds, and a final extension for 5 minutes at 75°C on an automated DNA thermal

cycler (Model 9700, Perkin-Elmer, Foster City, CA). PCR products were analyzed by electrophoresis on a 1.5 % agarose gel, stained with ethidium bromide (0.5 ug/ml), and photographed on a UV transilluminator.

Following electrophoresis, the DNA products were denatured in 0.5 N NaOH and  
5 1.5 M NaCl buffer for 30 minutes, transferred to a nylon membrane (Maximum Strength Nytran Plus, available from Schleicher & Schuell) overnight and cross-linked by exposure to UV light (UV Stratalinker 1800, available from Stratagene). The filters were incubated in prehybridization buffer (5 x SSC, 1X Denhardt's reagent, 0.2% SDS, 1 mg/ml sheared DNA) at 42°C for 2 hours and then in hybridization buffer (5 x SSC,  
10 1X Denhardt's reagent, 0.2% SDS, 1 mg/ml sheared DNA ) containing 5'  $\gamma$ -<sup>32</sup>P labeled oligonucleotide probe at 42°C overnight. After overnight incubation, membranes were washed twice in 2X SSC, 0.1% SDS for 15 minutes at room temperature, and then washed twice in 0.2X SSC, 0.1 % SDS at 55°C for 1 hour. The filters were autoradiographed at -70°C with Kodak XRR film.

15 Ethidium bromide-stained agarose gel and Southern hybridization analysis of PCR amplified products from oocyst-seeded fecal samples was performed in order to determine whether the dipstick method described herein resulted in a reduction of inhibition of PCR amplification of *T. gondii*-specific DNA in fecal slurries as compared with fecal slurries alone. Two sets of solutions, PBS and PBS/Feces (1:4 gm/ml), were  
20 seeded with four concentrations of oocysts,  $2 \times 10^6$ ,  $5 \times 10^5$ ,  $5 \times 10^4$ , and  $5 \times 10^3$ . Using the dipstick technique described above, this resulted in an estimated maximum number of oocysts in the PCR amplification tube to be 400, 100, 10, and 1 as indicated for the PBS solution and for the PBS/Feces solution respectively. Southern hybridization was performed using the OC-2 gene internal primer as the probe. Southern hybridization  
25 results and the ethidium bromide stained gel demonstrated that inhibition of PCR amplification of the exogenously added DNA was dramatically reduced (as compared with fecal extract alone) in samples prepared as per the dipstick assay as described above.

Three different paper supports were tested for their ability to support the PCR  
30 dipstick assay: IsoCodeJ™ Stix, S&S® #903™ (available from Schleicher and Schuell)

and Nobuto Blood Filter Strips (available from Advantec, Pleasantville, CA). First, IsoCodeJ™ Stix were tested for the ability to bind oocysts. Oocysts were diluted into either PBS or a suspension of uninfected feces and PBS. The fecal dipstick procedure as described above was used to sample and elute DNA for PCR analysis. The

5 concentration of oocysts per reaction was adjusted so that theoretical maximum could be 1, 10, 100, and 400 oocysts respectively. The amplification products were run on an agarose gel and stained as described above. According to this assay, oocysts diluted into PBS alone could be readily detected at 10 oocysts per ul of dipstick eluate with primers directed to the *T. gondii* OC-2 gene. In addition, oocysts in a suspension of feces and

10 PBS could be detected when present at a concentration of between 10 and 100 oocysts per ul. This experiment demonstrates that the oocysts are bound to the IsoCodeJ™ Stix in the presence of feces, are eluted by heat, and following a wash and heat elution step are sufficiently free from inhibitors to be detected by PCR amplification.

Under these conditions, detecting 10 oocysts per ul of eluate from the

15 IsoCodeJ™ Stix is equivalent to detecting oocysts at a concentration of  $2.5 \times 10^5$  oocysts/gram of feces. Several parameters were tested for their ability to increase the sensitivity of this test. First, two additional paper supports, S&S® #903™ and Nobuto Blood Filter Strips, were tested for both the ability to bind oocysts in the presence of solubilized feces, and the ability to support subsequent PCR detection of oocyst DNA.

20 Each of these filter papers bound *T. gondii* oocysts, and subsequent PCR amplification with OC-2 primers detected the presence of *T. gondii* DNA. However, the sensitivity of detection for each of these papers was somewhat less than the sensitivity of the assay when using IsoCodeJ Stix™. All three paper supports were also tested for binding of oocysts in the presence of feces over a range of pH from 4 to 9. The S&S® #903™ and

25 Nobuto Blood Filter Strips were most effective at pH 7. Binding of oocysts to the IsoCodeJ Stix™ was significantly increased at pH 9. All subsequent assays described below used IsoCodeJ Stix™ and pH 9 for binding of oocysts to dipsticks.

Another approach to increasing the sensitivity of the assay was to use primers from the *B1* gene during the PCR amplification reaction. The *B1* gene is a multicopy

30 gene that is present at approximately 35 copies per *T. gondii* genome. Using a *B1*-



specific primer resulted in a ten-fold increase in sensitivity, and produced an assay in which 1 oocyst/ul could routinely be detected. This level of sensitivity of the assay correlated with the ability to detect approximately  $1 \times 10^4$  oocysts/gram of feces.

The sensitivity and specificity of the PCR detection method was tested in experimentally infected animals using flotation and visualization of oocysts as the standard for quantification of oocysts. SPF cats were infected with mouse brain-derived tissue cysts and feces were collected from the cats for twenty-one days. Each sample was analyzed by both direct visualization and the dipstick PCR technique. Following gel electrophoresis of the products from PCR amplification, the results were scored as either positive or negative depending on the presence or absence of the correct gene-specific PCR product. Table 12 shows the results of PCR detection using both the *B1* and OC-2 DNA primers for each individual fecal sample. The positive and negative predicative values were 93.2% and 97.2% respectively using the *B1* gene DNA primers and 80.2% and 95.8% respectively using the OC-2 DNA primers.

TABLE 12. Sensitivity, specificity and predicative values for the PCR detection of oocysts in experimentally infected cat feces.

Method	Total Samples +/-	f/n <sup>a</sup>	f/p <sup>b</sup>	Sensitivity %	Specificity %	Predictive Value % +/-
Microscopy	69/176	0	0	100	100	100/100
PCR						
<i>B1</i> Primers	64/171	5	5	94.7	96.7	93.2/97.2
OC-2 Primers	61/161	7	16	89.7	96.4	80.2/95.8

<sup>a</sup> false negative

<sup>b</sup> false positive

#### Example 13:

A PCR ELISA was developed for the detection and quantification of PCR amplification products from the PCR dipstick method. In general, digoxigenin-labeled amplified product produced by the PCR dipstick detection method were detected by hybridization to an internal biotinylated *B1* gene primer bound to microtiter wells. The

concentration of PCR labeled digoxigenin fragment was determined using an alkaline phosphatase-linked anti-digoxigenin antibody (available from Boehringer Mannheim Biochemica GmbH). The alkaline phosphatase activity level was then determined using a standard ELISA reader. This quantitative PCR ELISA method detected oocysts at a lower limit of  $1 \times 10^4$  oocysts/gram when tested with uninfected cat feces seeded with known concentrations of *T. gondii* oocysts. The method is described in detail as follows.

PCR amplification using *B1* gene-specific primers was performed on eluates from the fecal dipstick method herein described. Amplification products were labeled by incorporation of digoxigenin-11-dUTP (DIG-11-dUTP) present in the reaction mix at 2.5  $\mu$ M. The concentration of dTTP in this reaction mix was reduced to 22.5  $\mu$ M. The resulting labeled fragment was detected using reagents from the PCR ELISA (DIG Detection) kit (available from Boehringer Mannheim Biochemica GmbH, Mannheim, Germany). The procedure was as follows. Four  $\mu$ l of the primary amplification reaction product was added to 16  $\mu$ l of denaturation buffer and incubated at room temperature for 10 minutes. This was mixed with 200  $\mu$ l hybridization buffer that contained 20 pmol/ml of the biotinylated *B1* gene probe. One-half of the hybridization reaction mixture was transferred to a well in a streptavidin-coated microtiter plate and incubated at 50°C for 3 hours with shaking. The plate was washed with washing buffer five times at room temperature and incubated with 100  $\mu$ l of anti-digoxigenin Fab conjugated with peroxidase at 37°C for 45 minutes. Following five washes, 100  $\mu$ l of ABTS substrate solution (available from Boehringer Mannheim Biochemica) was added to each well and the color was developed at room temperature for 45 minutes. The optical densities (OD) at 405 nm were read in a spectrophotometer (SpectraMAX 250, available from Molecular Devices Inc., Sunnyvale, CA) and analyzed with Soft Max Pro™ software (available from Molecular Devices Inc.).

Quantification of oocysts in feces by the PCR ELISA technique was compared with quantification by the microscopic analysis. Individual feces from six different cats were collected (as available) at various days post infection. Oocysts were then quantified for each sample by two separate techniques, microscopy and PCR ELISA.

The results from each of these two methods were in good agreement. Standard regression analysis produced a correlation coefficient of 0.91.

Example 14:

This example describes the detection of *Cryptosporidium parvum* oocysts and  
5 *Giardia lamblia* cysts in feces using the PCR dipstick detection method described above. Oocysts and cysts from *C. parvum* and *G. lamblia* respectively were detected by the dipstick PCR detection method, thereby demonstrating the usefulness of this method for the detection of cysts or oocysts from unrelated species.

Feline fecal samples from SPF cats were seeded with either *C. parvum* oocysts or  
10 *G. lamblia* cysts and used in the PCR detection method described herein. The primers used to detect *C. parvum* were specific for the *C. parvum* AWA gene, while the primers used to detect *G. lamblia* were specific for the *G. lamblia* ABB gene (Rochelle, et al., 1997, *Applied and Environmental Microbiology* 63:106-114).

In order to demonstrate binding of *C. parvum* oocysts to a dipstick in the  
15 presence of feline fecal slurry, aliquots of feline fecal slurry (1:4, mg/ml) were seeded with between  $5 \times 10^2$  and  $5 \times 10^6$  *C. parvum* oocysts/ml. These samples were then tested for binding of the oocysts and subsequent PCR analysis according to the PCR detection methods described herein. The primers used in the PCR amplification were specific for the *C. parvum* AWA gene. The PCR amplified products were run on an agarose gel and  
20 stained with ethidium bromide. The *C. parvum*-specific primer primed amplification of a DNA product of the predicted mobility, in an oocyst concentration-dependent manner, from the dipstick eluate as described above. The results of this experiment demonstrated that *C. parvum* oocysts bound to a dipstick in the presence of feline fecal slurry, and that about  $5 \times 10^2$  *C. parvum* oocysts/ml were detectable by the PCR detection method after  
25 binding to the dipstick under these conditions. Because  $5 \times 10^2$  oocysts/ml was the lowest concentration tested, and the products were easily observable, the concentration of cysts detectable by this method is likely to be lower than  $5 \times 10^2$  oocysts/ml.

In order to demonstrate binding of *Giardia* cysts to a dipstick in the presence of feline fecal slurry, aliquots of feline fecal slurry (1:4, mg/ml) were seeded with between  
30  $5 \times 10^2$  and  $5 \times 10^5$  *G. lamblia* cysts/ml. These samples were then tested for binding of

the cysts and subsequent PCR analysis according to the PCR detection methods described herein. The primers used in PCR amplification were specific for the *G. lamblia* ABB gene. The PCR amplified products were run on an agarose gel and stained with ethidium bromide. The *G. lamblia*-specific primer primed amplification of a DNA product of the predicted mobility, in a cyst concentration-dependent manner, from the dipstick eluate as described above. The results of this experiment demonstrated that *G. lamblia* cysts bound to a dipstick in the presence of feline fecal slurry, and that about  $5 \times 10^2$  *G. lamblia* cysts/ml were detectable by the PCR detection method after binding to the dipstick under these conditions. Because  $5 \times 10^2$  cysts/ml was the lowest concentration tested, and the products were easily observable, the concentration of cysts detectable by this method is likely to be lower than  $5 \times 10^2$  cysts/ml.

Example 15:

This Example discloses a method of isolation of *T. gondii* nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by intestinal secretions from infected cats. This Example further discloses recombinant nucleic acid molecules and proteins of the present invention.

The production of intestinal secretions and from infected cats and the use of these secretions for screening for nucleic acid molecules encoding immunogenic *T. gondii* proteins are described herein in Example 6. Intestinal secretions collected from a single cat that had been previously infected with *T. gondii* were pooled and preabsorbed to remove antibodies directed against UCG and *E. coli*. The pooled, preabsorbed intestinal secretions are also referred to herein as MGIS antiserum. MGIS antiserum was used to immune screen an ICG cDNA library in order to identify and isolate nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by intestinal secretions from infected cats. Six nucleic acid molecules encoding immunogenic *T. gondii* proteins recognized by intestinal secretions from infected cats were identified and isolated using the following methods. These six nucleic acid molecules are referred to herein as MGIS4-2 (also herein referred to as SEQ ID NO:282 and SEQ ID NO:284, representing the coding strand and its reverse complement, respectively), MGIS4-4 (also herein referred to as SEQ ID NO:292 and SEQ ID NO:294), MGIS4-8 (also herein

referred to as SEQ ID NO:306 and SEQ ID NO:308), MGIS6-5 (also herein referred to as SEQ ID NO:311 and SEQ ID NO:313), MGIS6-2 (also herein referred to as SEQ ID NO:326 and SEQ ID NO:328), and MGIS1-3 (also herein referred to as SEQ ID NO:329 and SEQ ID NO:331).

5        Absorption of MGIS Antibody

MGIS antiserum was collected, as previously described, from the cat intestine on weeks 6, 10, and 13 after infection, and on weeks 0, 1, 2, 3, 4, and 5 after challenge. Both pools of antisera were combined and used to screen the cDNA library, and are herein referred to as MGIS antiserum.

10        To remove anti-cat intestinal and anti-*E.coli* tissue reactive antibodies, the MGIS pools were absorbed to nitrocellulose (NC) filters coated with either cat intestinal proteins or *E.coli* proteins. Cat intestinal proteins used to coat the nitrocellulose filters were generated as follow. The epithelial layer of uninfected cat intestine was scraped on dry ice and the cells subsequently passed through several different gauge needles (No.  
15        18, 21, and 23) 10 times each. The sample was frozen and thawed 3 times, and then sonicated on ice for 10 minutes. The protein extract was diluted to 400ug/ml in PBS and immersed with the nitrocellulose at room temperature for 1 hour, and was then blocked with 4% milk in PBS for 30 minutes. Similarly, XL-1 blue *E.coli* cells were resuspended in PBS and bacterial protein extracts prepared similar to the cat intestinal  
20        proteins. The bacterial extract was diluted to a final concentration of 2.3 mg/ml in PBS and bound to the filter in a manner similar as the cat intestinal extract.

MGIS antiserum was diluted 1:20 with 4% milk in PBS and absorbed sequentially to both the cat intestinal and bacterial protein coated filters at room temperature for 1 hour. To demonstrate that all UCG and *E. coli*-reactive antibody had  
25        been removed from the MGIS antiserum preparation, the MGIS antiserum subjected to Western blot analysis which showed that the absorbed antibody had no reactivity to either the cat intestinal proteins or to the bacterial extract.

Immune Screening of *T. gondii* cDNA Phage Library

The ICG cDNA library was constructed from infected cat intestinal mRNA, and  
30        the cDNA product cloned into the *EcoRI/XhoI* sites of the Uni-Zap XR vector.

*Toxoplasma*-specific nucleic acid molecules represented approximately 10% of the library. The ICG cDNA phage library was plated to approximately  $2.5 \times 10^{4-5}$  pfu per 135 mm plates with XL-1Blue MRF' cells (available from Stratagene). Ten plates were treated in the following manner after the phage were pinhead in size. Nitrocellulose filters that had been previously treated with IPTG were overlaid on top of the phage and incubated at 37°C for 5 hours. The filters were marked, washed with TBS, pH 8.0, blocked with 4% milk in TBS, and incubated with MGIS antiserum at room temperature overnight. After washing three times with TBS, horse-radish peroxidase (HRP)-labeled goat anti-cat IgA antibody (Bethyl Lab. Inc.) was diluted 1:350, and incubated with the filters at room temperature for 2 hours. The color indicator was developed with 4-chloro-1-naphthol substrate and  $H_2O_2$ . Forty-one positive clones were selected for further screening.

#### Hybridization Screening and Clone Purification

Selected clones were replated on NZYM plates, and forty-eight individual plaques randomly picked and resuspended in 100 ul of SM buffer. Insert DNAs were PCR amplified in a final volume of 12.5 ul containing 1ul of template DNA, 50mM KCL, 10mM Tris-HCL (pH 8.3), 2mM  $MgCl_2$ , 0.2mM each dNTP, 0.2mM each of T3 and T7 vector specific oligonucleotide primers, and 0.3 units of Taq polymerase. Amplification was performed by 1 cycle of 95° C for 3 min., 35 cycles of 95° C for 30 sec., 50° C for 30 sec., and 72° C for 2 min., followed by 75° C for 5 min. on a Perkin Elmer 9600 thermocycler. The PCR amplified products were analyzed on a 1% agarose TBE gel, and the DNA transferred to a nylon membrane.

A hundred nanograms of *T. gondii* genomic DNA was labeled using the Megaprime DNA labeling systems (available from Amersham International) and used as a probe to analyze the PCR amplified DNA fragments on the nylon membrane. The membrane was pre-hybridized in 5xSSPE (1x SSPE: 0.18M NaCl, 10mM  $NaH_2PO_4$ , and 1mM EDTA pH 7.7), 0.5% SDS, 5x Denhardt's solution, and 0.1mg/ml single stranded salmon sperm DNA at 65° C for 3 hours. Membranes were then hybridized overnight at 65° C, and then washed with 2xSSPE, 0.1% SDS at room temperature for 10 min., twice, and 0.2xSSPE, 0.5% SDS at 65° C for 1 hour, twice. The membrane was exposed to

film at -70° C overnight. Twenty-three clones were thus shown to contain *T. gondii*-specific DNA, with an insert size of 1-2 Kb in length.

#### Clone Identification by Phage Drop Test

Each of the twenty-three *T. gondii*-specific clones were rescreened to confirm reactivity with MGIS antiserum. Phage clones were diluted 1:10<sup>6</sup> from the SM buffer stock, and 3ul of this dilution (~5-50 phage) was spotted onto a NZYM/XL-1Blue MRF' agar plate, and incubated at 37° C for 5 hours. Afterwards, an IPTG pre-treated nitrocellulose filter was overlaid onto the agar surface and incubated for another 5 hours. The filter was marked, washed with TBS buffer (pH 8.0) at room temperature for 15 minutes, and blocked with 4% milk in PBS for 30 minutes. Pre-absorbed MGIS antiserum was added to the filter and allowed to react at room temperature overnight. The filter was subsequently washed in TBS at room temperature for 10 minutes, three times. Goat anti-cat IgA polyclonal antibody labeled with HRP (available from Bethyl Laboratories, Inc.) was diluted 1:300 in TBS buffer and incubated with the filter at room temperature for 2 hours. The filter was washed and developed using 4-chloro-1-naphthol substrate and H<sub>2</sub>O<sub>2</sub>. Thirteen of the 23 clones were identified as positive for expressing antigen recognized by IgA in the MGIS antiserum.

#### DNA Sequencing

The DNA inserts in the thirteen clones identified as positive were subcloned into the TA vector using the TA cloning kit (available from Invitrogen). Individual clones were PCR amplified using the T3 and T7 vector-specific primers. The DNA fragments produced by PCR amplification were gel electrophoresed on a 1% agarose gel, and gel purified using a Qiagen Gel Purification kit (available from Qiagen). Plasmid DNA was purified using the 5 prime 3 prime Perfect Plasmid DNA Preparation kit (available from 5 Prime 3 Prime Inc., Boulder, CO). DNA sequencing was carried out on six of the *T. gondii*-specific DNA inserts using a Prizm dideoxy termination kit (available from Perkin Elmer) on an ABI 377 DNA sequencer (available from Applied Biosystems). TA sense and TA antisense oligonucleotide primers were used for DNA sequencing, and insert-specific oligonucleotide primers were used to generate internal fragment sequences. The only variation from this general protocol was in the case of MGIS4-4,

where the Erase a Base system (available from Promega) was used to generate plasmids containing deleted fragments in order to facilitate sequencing. The primers used for sequencing each of the inserts were the following:

The primers used in sequencing MGIS4-2 are herein referred to as SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, SEQ ID NO:279, SEQ ID NO:280, and SEQ ID NO:281. The primers used in sequencing MGIS4-4 are herein referred to as SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, and SEQ ID NO:291. The primers used in sequencing MGIS4-8 are herein referred to as SEQ ID NO:295, SEQ ID NO:296, SEQ ID NO:297, SEQ ID NO:298, SEQ ID NO:299, SEQ ID NO:300, SEQ ID NO:301, SEQ ID NO:302, SEQ ID NO:303, SEQ ID NO:304, and SEQ ID NO:305. The primers used in sequencing MGIS6-5 are herein referred to as SEQ ID NO:309 and SEQ ID NO:310. The primers used in sequencing MGIS6-2 are herein referred to as SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, and SEQ ID NO:325. And the primers used in sequencing MGIS1-3 are herein referred to as SEQ ID NO:314, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:319, SEQ ID NO:320, SEQ ID NO:321, SEQ ID NO:322, SEQ ID NO:323, SEQ ID NO:324, and SEQ ID NO:325 (note that the same primers were used for sequencing MGIS6-2 and MGIS1-3).

#### PCR Amplification of Feline and *T. gondii* DNA With Clone-specific Primers

The IgA selected MGIS clones were shown to be *Toxoplasma* specific by PCR amplification analysis. The following different cDNA samples were tested for the presence of DNA representing each of the six different IgA-selected nucleic acid molecules: a) uninfected cat gut (UCG); b) infected cat gut (ICG); c) *T. gondii* tachyzoite (TgTz); d) *Toxoplasma* bradyzoite (TgBz); and e) *Toxoplasma* genomic DNA (TgTz DNA). The preparation of UCG, ICG, *Toxoplasma* tachyzoite and bradyzoite cDNA was as described above. *Toxoplasma* genomic DNA was isolated from tachyzoites by phenol/chloroform/isoamylalcohol pH 8.0 extraction.



Oligonucleotide sense and anti-sense primers specific to each of five MGIS-selected nucleic acid molecules were synthesized and used as primers in the PCR amplification reactions. The reaction condition were: 95 °C for 10 min., followed by 35 cycles of 95 °C for 30 sec., 58 °C for 30 sec., 72 °C for 40 sec; this was followed by 75°C for 5 min.

- 5 afterwards to complete the reaction. The amount of the different templates used in the PCR reactions ( ~3-30 ng of DNA) , was empirically determined by comparison with a PCR amplified *Toxoplasma* tubulin gene product standard generated with each template. The oligonucleotide primers and the size of the expected products are listed in Table 13, below.

10 Table 13.

MGIS Clone	Sense Primer Position: Sequence	Anti-Sense Primer Position: Sequence	Product Size (bp)
1-3	1513: SEQ ID NO: 319	1858: SEQ ID NO: 320	346
4-2	168: SEQ ID NO: 276	594: SEQ ID NO: 279	427
15 4-4	455: SEQ ID NO: 285	775: SEQ ID NO: 290	331
4-8	2018: SEQ ID NO: 300	2310: SEQ ID NO: 301	293
6-2	1301: SEQ ID NO: 319	1646: SEQ ID NO: 320	346

- The oligonucleotide primers specific for each of the five MGIS-selected nucleic acid molecules PCR amplified products only when the template DNA contained *Toxoplasma* DNA. There were no PCR amplified products in this assay when the template DNA was UCG cDNA. These results confirm the *T. gondii* origin of the MGIS-selected nucleic acid molecules.

#### Sequence Analysis

- Homology searches of a non-redundant protein database were performed on all six MGIS-selected nucleic acid molecules, translated into all six reading frames, using the BLASTX program available through the BLAST™ network of the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, MD). This database includes SwissProt + PIR + SPupdate + GenPept + GPUupdate + PDB databases. In addition, BLASTN homology searches were

performed on these sequences using the NCBI databases including the non-redundant database of GenBank EST, and geneml. In all cases, the default parameters for the homology programs were used.

The highest scoring match of the homology search (BLASTX) of translation products of the nucleic acid sequence SEQ ID NO:282 (MGIS4-2) was to GenBank™ Accession No. prf 2208369A, a *Homo sapiens* signal peptidase 12kD subunit protein. The protein encoded by nucleic acid residues 742-945 of MGIS4-2 (SEQ ID NO:282) showed about 44% identity to amino acid residues 12 to 79 of the protein represented by GenBank™ Accession No. prf 2208369A. At the nucleotide level, SEQ ID NO:282 showed 97% identity over 353 nt with the sequence represented by GenBank™ Accession No. W0680 (TgESTzy81e12.r1), an EST fragment isolated from *T. gondii* tachyzoite cDNA. The homology spans the region from nt 748 to nt 1097 of SEQ ID NO:282, and nt 15 to 365 of GenBank™ Accession No. W0680. There were no other significant homology matches to SEQ ID NO:282 nucleic acid sequence.

The highest scoring matches of the homology search (BLASTX) of translation products of the nucleic acid sequence SEQ ID NO:292 (MGIS4-4) were to proteins described as elongation factor 1-gamma, with the highest match to the sequence represented by GenBank™ Accession No. gi 2160158, described as "a protein similar to elongation factor". The protein encoded by residues 47-1222 of SEQ ID NO:292 showed about 37% identity to amino acid residues 5-414 of the protein represented by GenBank™ Accession No. gi 2160158. At the nucleotide level SEQ ID NO:292 showed 94% identity over 413 nt with an EST fragment, GenBank™ Accession No. N81326 (TgESTzy40a12.r1), an EST fragment isolated from *T. gondii* cDNA. The homology spans the region from nt 420 to nt 832 of SEQ ID NO:292, and nt 15 to 427 of GenBank™ Accession No. N81326. In addition, SEQ ID NO:292 showed 99% identity over 187 nt with an EST fragment, GenBank™ Accession No. W05869 (TgESTzy85a09.r1), an EST fragment isolated from *T. gondii* cDNA clone. The homology spans the region from nt 757 to nt 943 of SEQ ID NO:292, and nt 62 to 248 of GenBank™ Accession No. W05869.

The highest scoring match of the homology search (BLASTX in the genembl database) of translation products of the nucleic acid sequence SEQ ID NO:329 (MGIS1-3) was to Herpesvirus Saimiri complete genome, represented by GenBank™ Accession No. X64346. The amino acid residues 777 to 1432 of the protein encoded by reading  
5 frame +2 of SEQ ID NO:329 showed about 36% identity to amino acid residues 106974 to 106517 of the protein represented by GenBank™ Accession No. X64346. At the nucleotide level, SEQ ID NO:329 showed 94% identity over 578 nt with an EST fragment, GenBank™ Accession No. AA520348 (TgESTzz69d04.r1), an EST fragment isolated from *T. gondii* bradyzoite cDNA. The homology spans the region from nt 1334  
10 to 1910 of SEQ ID NO:329, and nt 5 to 571 of GenBank™ Accession No. AA520348.

The highest scoring match of the homology search (BLASTN of the non-redundant databases, GenBank+EMBL+DDBJ+PDB) of SEQ ID NO:311 (MGIS6-5) was to a *T. gondii* lactate dehydrogenase gene, represented by GenBank™ Accession No. TGU35118. SEQ ID NO:311 showed 99% identity over 1619 nt.

15 The highest scoring match of the homology search (BLASTX in the genembl database) of translation products of the nucleic acid sequence SEQ ID NO:326 (MGIS6-2) was to Herpesvirus Saimiri complete genome, represented by GenBank™ Accession No. X64346. Amino acid residues 751 to 1206 encoded by SEQ ID NO:326 showed about 36% identity to amino acid residues 106972 to 106517 of the protein represented  
20 by GenBank™ Accession No. X64346. At the nucleotide level, SEQ ID NO:326 showed 96% identity over 247 nucleotides with an EST fragment, GenBank™ Accession No. AA520348 (TgESTzz69d04.r1), an EST fragment isolated from *T. gondii* bradyzoite cDNA. The homology spans the region from nt 890 to 1136 of SEQ ID NO:326, and nt 144 to 390 of GenBank™ Accession No. AA520348.

25 The highest scoring match of the homology search (BLASTX of the non-redundant GenBank CDS database including Translations+PDB+SwissProt+SPupdate+PIR) of translation products of the nucleic acid sequence SEQ ID NO:306 (MGIS4-8) was to a rice 26S protease regulatory subunit 4 homolog (TAT-binding protein homolog 2), represented by GenBank™ Accession No.  
30 P46466. 26S protease regulatory subunit 4 homologs representing other species also

have high homology to a translation product of SEQ ID NO:306. The protein encoded by nucleic acid residues 465 to 1565 of SEQ ID NO:306 showed about 72% identity to amino acid residues 35 to 448 of the protein represented by GenBank™ Accession No. X64346. It should be noted a gap of 42 amino acids was required in the amino acid  
5 sequence encoded by SEQ ID NO:306 in order to achieve the sequence fit resulting in this high homology. At the nucleotide level, SEQ ID NO:306 showed 98% identity over 269 nucleotides with an EST fragment, GenBank™ Accession No. W35531 (TgESTzy90g01.r1), an EST fragment isolated from *T. gondii* cDNA. The homology spans the region from nt 668 to nt 936 of SEQ ID NO:326, and nt 23 to nt 291 of  
10 GenBank™ Accession No. W35531.

Example 16:

This Example discloses the isolation and sequence analysis of a 1397 bp *T. gondii* nucleic acid molecule composed of four fragments isolated by subtractive selection from an infected cat gut cDNA library. Also described is an additional nucleic  
15 acid molecule representing the genomic DNA sequence immediately upstream (5') of, and overlapping, the genomic DNA sequence encoding the cDNA sequence.

A 1397 bp *T. gondii* nucleic acid molecule, denoted nTG<sub>1397</sub> (the coding strand of which is herein referred to as SEQ ID NO:343, and the reverse complement of which is herein referred to as SEQ ID NO:345), is a composite of four overlapping PCR  
20 amplified products isolated from an infected cat gut (ICG) cDNA library. Specifically, a first 424 bp fragment (representing nucleotide positions 709-1132 of SEQ ID NO:343), was isolated after two rounds of selection using the PCR-Select™ Subtraction kit (available from Clontech, Palo Alto, CA), using day eight, *RsaI* restriction enzyme digested ICG cDNA as tester, and similarly digested uninfected cat gut cDNA as driver  
25 DNA. Fragments enriched by the PCR-Select™ Subtraction selection process were digested with the restriction enzyme *SmaI* and cloned into *SmaI* site in the commercially available positive selection vector, QuanTox™ (available from Quantum Biotechnologies Inc., Laval, Quebec, Canada). The cloned inserts were subsequently sequenced using the oligonucleotide primers, T7 (TAATACGACTCACTATAGGG, 30 herein referred to as SEQ ID NO:348) and T3 (ATTAACCCTCACTAAAGGGA, herein

referred to as SEQ ID NO:347). A 424 bp *T. gondii* nucleic acid molecule, referred to herein as nTG<sub>424</sub>, was isolated, cloned and sequenced by this method.

The orientation of nTG<sub>424</sub>, as well as additional nucleic acid sequence representing cDNA sequence occurring downstream (3') of nTG<sub>424</sub> was determined as follows. A 689 bp fragment including the 3'-end of the gene comprising nTG<sub>424</sub> was generated by PCR amplification of an ICG cDNA library constructed in the Uni-Zap XR insertion vector (available from Stratagene). The two primers used for this amplification reaction are represented by SEQ ID NO:358 (709ACAACGACCACGACATCAACTAC<sup>731</sup>, derived from the sequence of nTG<sub>424</sub>, also referred to as pRay8), and an adaptor oligonucleotide primer that hybridized to the cDNA poly A tail (GGCCACGCGTCGACTACT<sub>17</sub> from BRL/GIBCO, Gaithersburg, MD, herein referred to as SEQ ID NO:364). The superscript numbers at the beginning and end of the primer sequences described herein represent the location of the primer sequence relative to nTG<sub>1397</sub> (SEQ ID NO:343). A resulting 689bp *T. gondii* nucleic acid molecule (also referred to as nTG<sub>689</sub>) was cloned into PCR2.1 (available from Invitrogen, Carlsbad, CA), and sequenced using the M13 reverse oligonucleotide primers (CAGGAAACAGCTATGACC, herein referred to as SEQ ID NO:346) and the T7 oligonucleotide primer (SEQ ID NO:348). The sequence of nTG<sub>689</sub> revealed 266 bp of additional cDNA sequence (from 1133-1397 bp, relative to SEQ ID NO:343), with an overlap with nTG<sub>424</sub> from 709-1132 bp (relative to SEQ ID NO:343). There were three nucleotide differences between the sequence data for nTG<sub>424</sub> and the sequence data for nTG<sub>689</sub>. Instead of a "T", "C" and "T" nucleotide at positions 1159, 1166, and 1169 respectively, the sequence data for nTG<sub>689</sub> revealed a "C", "T", and "A" at those positions.

The remainder of the nucleic acid sequence of nTG<sub>1397</sub> was determined in two PCR amplification steps using the ICG cDNA library as the template. The primers for the first PCR amplification were: a) an anti-sense oligonucleotide primer specific for nTG<sub>424</sub>, having the sequence <sup>929</sup>GTTGTCGTAGATGTCGTTGTAGTT<sup>906</sup>, and herein referred to as SEQ ID NO:359; and b) a Uni-Zap XR insertion vector-specific oligonucleotide primer (available from Stratagene, and referred to as Tp277) having the

sequence, GGGAACAAAAGCTGGAGCTCCACC, and herein referred to as SEQ ID NO:354. In the first PCR amplification step, SEQ ID NO:359 and SEQ ID NO:354 were used to generate an 884 bp nucleic acid molecule, (825 bp of which was nTG<sub>1397</sub>-specific DNA sequence), that was then cloned into PCR2.1. The *T. gondii*-specific nucleic acid molecule is herein referred to as nTG<sub>825</sub>. nTG<sub>825</sub> was sequenced using a TA sense oligonucleotide primer (having the sequence, CGAGCTCGGATCCACTAG, herein referred to as SEQ ID NO:350), and a TA anti-sense oligonucleotide primer (having the sequence, GCCAGTGTGATGGATATCTGCAG, herein referred to as SEQ ID NO:349), as well as a nTG<sub>1397</sub>-specific internal oligonucleotide primer having the sequence, <sup>564</sup>GAGGAGATCGAACTTTGCTTGTGC<sup>541</sup>, herein referred to as SEQ ID NO:361. Sequencing revealed that nTG<sub>825</sub> added an additional 604 bp to the sequence of nTG<sub>1397</sub>, from nucleotides 105-708 (relative to SEQ ID NO:343). nTG<sub>825</sub> overlapped with nTG<sub>424</sub> and nTG<sub>689</sub> from base 709-939 (relative to SEQ ID NO:343).

The primers for the second PCR amplification step were: a) an oligonucleotide primer specific for nTG<sub>424</sub>, having the sequence <sup>225</sup>AGAAGCGCCTTTGCGTTTCTACGT<sup>202</sup>, herein referred to as SEQ ID NO:360; and b) Tp277. These two primers were used to generate a 225 bp *T. gondii* DNA fragment, referred to as nTG<sub>225</sub>. nTG<sub>225</sub> cloned into PCR2.1, and nucleotide sequenced with the TA oligonucleotide primers as above, thereby generating the sequence from nucleotides 1-104 of SEQ ID NO:343. Sequence analysis revealed that nTG<sub>225</sub> overlapped with previously isolated nTG<sub>825</sub> DNA sequence from base 105-225, relative to SEQ ID NO:343.

The contiguous cDNA sequence of the overlapping fragments representing nTG<sub>1397</sub> was determined (and referred to herein as SEQ ID NO:343), and sequence analysis of the composite molecule revealed an 867 bp coding region (referred to as nTG<sub>867</sub>), assuming an initiation codon at position 238-240, and a stop codon at position 1102-1104 (relative to SEQ ID NO: 343). The coding strand of nTG<sub>867</sub> is herein referred to as SEQ ID NO:340, and the reverse complement is herein referred to as SEQ ID NO:342. Translation of the coding region of nTG<sub>867</sub> yields a 288 amino acid protein

herein referred to as PTg<sub>288</sub>, the amino acid sequence of which is herein referred to as SEQ ID NO:341.

To confirm the DNA sequence in the predicted coding region of nTG<sub>1397</sub>, a PCR amplified fragment containing nucleotides 238 to 1271 was generated using an  
5 oligonucleotide primer having the sequence, *AAGGATAGGCGGCCGCAGGTACC*  
<sup>238</sup>*ATGGCAGGAAGGCAGGCGGCGTT*<sup>260</sup>, herein referred to as SEQ ID NO:362, and  
an oligonucleotide primer having the sequence, *ACCGCTCGAGAAGCTT*  
<sup>1271</sup>*GAAGCCAAGACATCCCTTCGTGCA*<sup>1248</sup>, herein referred to as SEQ ID NO:363.  
The nucleotides in italics represent non-nTG<sub>1397</sub> nucleotide sequence, and were present to  
10 attach convenient restriction sites to the PCR product. The resulting PCR fragment was  
cloned into a eukaryotic expression vector, referred to as pDVacIII, and sequenced using  
two vector-specific oligonucleotide primers: a) Tp244, having the sequence,  
*GGATGCAATGAAGAGAGGGCTC*, and herein referred to as SEQ ID NO:352; and  
b) Tp245, having the sequence, *AACTAGAAGGCACAGTCGAGGCTG*, and herein  
15 referred to as SEQ ID NO:353. The PCR fragment thus generated contained two  
nucleotide differences as compared with the previously determined cDNA sequence of  
nTG<sub>1397</sub>. Instead of an "A" at position 643, a "G" residue was found, and in place of a  
"T" at position 1187, a "C" residue was found. The resulting nucleotide change at  
position 643 altered the predicted encoded amino acid from an arginine to a glycine  
20 residue. The change at position 1187 did not change the predicted amino acid sequence  
of nTG<sub>1397</sub>.

Genomic DNA sequence upstream of the gene comprising nTG<sub>1397</sub> was  
determined by generating a 747 bp fragment by PCR amplification of the  $\lambda$ -EMBL-3  
*Sau3A* partial *Toxoplasma* genomic library herein described. The primers used were  
25 SEQ ID NO:360 (representing nucleotides 202-225 in nTG<sub>1397</sub>) and a  $\lambda$ -EMBL-3-  
specific primer having the sequence, *GGTTCTCTCCAGAGGTTTCATTAC*, and herein  
referred to as SEQ ID NO:351. The resulting DNA fragment was cloned in PCR2.1 and  
sequenced with TA oligonucleotide primers (SEQ ID NO:349, and SEQ ID NO:350) and  
two gene specific oligonucleotide primers, Tp310  
30 (<sup>365</sup>*CGGACGTTGCATGTCAGTGGACA*<sup>343</sup>, herein referred to as SEQ ID NO:355) and

Tp311 (<sup>243</sup>CACGAAGCTGCATGTTCCAGCTAG<sup>265</sup>, herein referred to as SEQ ID NO:356). The sequence of the PCR fragment revealed a 647 bp DNA fragment, nTG<sub>647</sub>, (herein referred to as SEQ ID NO:338, the reverse complement is herein referred to as SEQ ID NO:339, including 421 nucleotides of new genomic DNA sequence upstream of the 5' end of the cDNA sequence of Tg<sub>1397</sub>. The fragment contained 327 bp of genomic DNA sequence that overlapped with the cDNA sequence, SEQ ID NO:343 (in other words, bases 422-647 of the genomic DNA sequence, SEQ ID NO:338, overlap with bases 1-225 of the cDNA sequence, SEQ ID NO:343). There was a single nucleotide difference between the genomic and the cDNA sequences at position 118 of the cDNA sequence (SEQ ID NO:343), where there is a "G" in the genomic DNA sequence and an "A" at the equivalent position in the cDNA sequence.

SEQ ID NO:343 was shown to be *T. gondii* specific by PCR amplification analysis of various DNAs, using nTG<sub>1397</sub>-specific DNA primers to drive the reaction. The following cDNA samples were tested for the presence of nTG<sub>1397</sub> DNA: a) uninfected cat gut (UCG), b) infected cat gut (ICG), c) *T. gondii* tachyzoite (TgTz), and d) *Toxoplasma* bradyzoite (TgBz). To generate UCG and ICG RNA, gut tissue samples from an uninfected cat and a cat 7 days post infection with *T. gondii* tissue cysts (1000 cysts) were processed by scraping and collecting the epithelial layer of gut cells on dry ice. Cells from UCG, ICG, and *T. gondii* tachyzoites and bradyzoites were solubilized by homogenization in TRI-reagent (available from Molecular Research Center Inc., Cincinnati, OH), and the homogenate passed through a 18/20/and 22 gauge needle 10 times each sequentially. After standing at room temperature for 5 min., 100 ul of bromochloropropane (available from Molecular Research Center Inc.)/ ml of TRI reagent was added, and the homogenate vortexed for 15 seconds. The sample was centrifuged at 14,000 rpm for 15 min. at 4°C, the aqueous layer collected, and RNA precipitated with one half volume of isopropanol. Contaminating genomic DNA was removed by digestion with 10 units of RNase free DNaseI (available from Boehringer Mannheim Corp.) at 37 °C for 30 min. The sample was then extracted with phenol/chloroform/isoamylalcohol, pH 6.0. The RNA was precipitated from the aqueous layer with ethanol and resuspended in diethylpyrocarbonate (available from



Sigma) treated water. cDNA was generated from total RNA using a commercially available RT-PCR kit (available from Stratagene).

Two nTG<sub>1397</sub>-specific oligonucleotide primers were used in the reaction: SEQ ID NO:358, having the sequence, <sup>709</sup>ACAACGACCACGACATCAACTAC<sup>731</sup>, and SEQ ID NO:357, having the sequence, <sup>1114</sup>ACACTTTGGTCTAATCGAGGGTAG<sup>1091</sup>. The reaction conditions were: 95 °C 12 min., followed by 3 cycles of 94 °C 30 sec., 70 °C 30 sec., 72 °C 60 sec., 3 cycles of 94 °C 30 sec., 67 °C 30 sec., 72 °C 60 sec., 3 cycles of 94 °C 30 sec., 65 °C 30 sec., 72 °C 60 sec., 6 cycles of 94 °C 30 sec., 63 °C 30 sec., 72 °C 60 sec., 25 cycles of 94 °C 30 sec., 59 °C 30 sec., 72 °C 60 sec., and a seven minute extension at 75 °C to complete the reaction. The amount of template used in each PCR reaction (~3-30 ng of DNA), was empirically determined by comparison with a PCR amplified *Toxoplasma* tubulin gene product standard generated with each template. The PCR amplification reaction generated a 406 bp product only in the reactions containing tachyzoite and ICG cDNA template DNA, thereby confirming the *T. gondii*-specificity of SEQ ID NO:343.

#### Sequence Analysis

Homology searches of a non-redundant protein database were performed on SEQ ID NO:340 (representing the coding region of nTG<sub>1397</sub>, translated in frame 1, using the BLASTP program available through the BLAST™ network of the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, MD). This database searched was PIR. In addition, a BLASTP homology search was performed on SEQ ID NO:341 (representing the amino acid sequence encoded by SEQ ID NO:340) using the NCBI database SwissProt. In all cases, the default parameters for the homology programs were used. Another homology search was run on SEQ ID NO:343 using the BLASTN search program and the database genembl.

When run against the PIR database, the highest scoring match of the homology search of translation products of the nucleic acid sequence SEQ ID NO:340 (the coding strand of the coding sequence) was to GenBank™ accession number A60095, a *Drosophila* larval glue protein precursor. Other significant homologies included

homology to an African clawed frog mucin, and a promastigote surface antigen-2.

When analyzed by the GCG program, using BESTFIT and default parameters, amino acid residues 145 to 281 of the protein encoded by SEQ ID NO:340 showed about 70% identity to amino acid residues 42 to 178 of the protein represented by GenBank™

- 5 accession number A60095. In addition, amino acid residues 153 to 282 of the protein encoded by SEQ ID NO:340 showed about 73% identity to amino acid residues 394 to 523 of the protein represented by GenBank™ accession number A45155 (African clawed frog mucin). When compared with the SwissProt database, the highest scoring match of the homology search of the amino acid sequence SEQ ID NO:341 (the protein
- 10 encoded by SEQ ID NO:340) was to GenBank™ accession number Q05049, the African clawed frog mucin. These two amino acid sequences showed a 73% identity from amino acid 153 to 282 of SEQ ID NO:341 and amino acid 394 to 523 of the amino acid sequence represented by GenBank™ accession number Q05049. A comparison of SEQ ID NO:343 (the cDNA coding strand) using the BLASTN search program and the
- 15 database geneml revealed a 76% nucleic acid sequence identity to a *D. discoideum* protein kinase, GenBank™ accession number M38703. This identity was between nt 765 to 1058 of SEQ ID NO:343 and nt 772 to 1065 of the sequence represented by GenBank™ accession number M38703. In addition, a BLASTN comparison SEQ ID NO:343 with the non-redundant GenBank™ database including GenBank
- 20 EMBL+DDBJ+PDB revealed an 89% identity between nucleic acid residues 779 to 902 of SEQ ID NO:343 and nt 2150 to nt 2273 of the nucleic acid sequence represented by GenBank™ accession number DDDU86962.

Example 17:

- This example describes the induction of humoral and cellular responses in cats by
- 25 proteins expressed by the *T. gondii* nucleic acid molecules of the present invention. Protein immunization with *T. gondii* recombinant protein and several different adjuvants induced both antibodies and T cell proliferative responses in cats. DNA immunization of cats with plasmid constructs expressing *T. gondii* immunogenic proteins of the present invention also induced antibody responses.

### Protein Immunization

Protein immunization of cats was carried out with three primary subcutaneous immunizations at intervals of four weeks (prime at week 0 and boosts at weeks 4 and 8) using 50 µg protein per injection in adjuvant. The primary antigen was OC-22, which  
5 was purified as a HIS fusion protein from *E. coli*. The experimental groups were as follows: two cats were immunized with OC-22 protein in alum, two cats were immunized with OC-22 protein in polyphosphazine (PCPP), and two cats were immunized with OC-22 protein in BAYER1005 (Stunkel, K.G., et al., in *Cellular Basis of Immune Modulation*, 1989, pp. 575-579, incorporated herein by reference in its  
10 entirety). One cat was injected with two different antigens in BAYER1005: 50 µg of OC-22 and 12 µg of protein 4499-9. One control cat was injected with saline.

Whole blood was collected from all of the animals at intervals before and after the immunizations. Mononuclear cells were selected from the blood for T cell proliferation analysis (see blow) and the remaining plasma processed for detection of  
15 humoral responses. The presence of antibody was determined by western blot analysis and by ELISA using recombinant purified antigens. The western blot analysis was more sensitive at detecting a positive or negative response, while the ELISA provided a more quantitative comparison of the cat's responses to the immunogenic proteins.

Western blot analysis was performed on Recombinant purified OC-22 protein  
20 was loaded at 2 µg per lane and blotted to nitrocellulose. Samples were from pre-immune cats and cats at 1, 3, and 5 weeks after immunization. Recombinant purified OC-22 protein was loaded at 2 µg per lane and blotted to nitrocellulose. Analysis of the sera collected at three weeks following the first immunization demonstrated that all seven cats responded positively to OC-22 protein. Both anti-cat IgG and anti-cat IgA  
25 were used as secondary antibodies (on separate blots). The westerns showed that OC-22 protein elicited both IgA and IgG responses, although the IgA response was not as strong as the IgG response. The ELISA titers were monitored throughout the immunization regimen. The sera collected at week eight and a half, immediately following the second boost had detectable ELISA titers equal to or greater than 1:10,000 for all seven cats.  
30 These analyses did not demonstrate any apparent differences between the cats immunized with different adjuvants. The single cat immunized with 12 µg of 4499-9

protein was not positive to 4499-9 protein by either western blot analysis or ELISA, although the same cat demonstrated immune responses to OC-22 that were comparable to the other cats in the study.

Cellular responses to the recombinant *T. gondii* OC-22 protein were tested by *in vitro* proliferation of isolated peripheral blood mononuclear cells (PBMC) to purified protein at concentrations ranging from 0.5 to 8 µg /ml. At higher concentrations of protein, non-specific stimulation was evident, making interpretation difficult, but at lower concentrations of antigen, distinct differences were seen between cats. One week after the first boost, T cells from all of the cats in either the PCPP or BAY R1005 adjuvant groups demonstrated stimulation indices (SI) greater than 3. Cells from the PBS control and two alum group cats did not show any proliferative responses. Peak proliferative responses were seen one week after each boost, with the highest responses observed after the first boost. The cats immunized with protein in PCPP had the highest responses, followed by the cats immunized with protein in BAY R1005. The responses observed at 0.5 µg antigen per ml were lower than the responses observed at higher doses, but correlated well with the results observed at 2 ug/ml (data not shown). All of the immunized cats responded to antigen, at some point during the experiment, with an SI level above 3.

#### DNA Immunization

Cats were immunized with the recombinant eukaryotic expression vector, pDVac II, encoding *T. gondii* nucleic acid molecules encoding the immunogenic proteins OC-2, OC-22, and Tg-50. The pDVacII vector contains the CMV promoter and intron A sequences. The protein expressed by this vector includes the *T. gondii* antigen of interest, fused at the 5 prime end to the tissue plasminogen activator signal sequence and fused at the three prime end with both a stretch of poly histidines and an amino acid epitope from the mammalian *myc* gene. Fifteen cats were divided into four experimental groups: three cats received saline (cats 1, 8, and 16), four cats received DNA encoding OC-2 (cats 2, 5, 9, and 15), four cats received DNA encoding OC-22 (cats 3, 6, 10, and 12), and four cats received a combination of DNA encoding OC-2, OC-22, and Tg-50 (cats 4, 7, 13, and 14). Each cat was injected intramuscularly with a total 300 µg of DNA at two sites per immunization. The combined formulation included 300 µg of each

plasmid per injection. The cats were given one injection and then at eight weeks received a boost.

The serum samples collected at six weeks after the primary immunization were analyzed. Two out of eight cats immunized with OC-2 DNA were shown to sero convert to antibody positive to OC-2 protein by western blot analysis. None of the sera collected at this time from the cats immunized with OC-22 or Tg-50 DNA were positive by western blot analysis to OC-22 or Tg-50 protein respectively. When sera collected one week following the boost (week 9) were analyzed by western blots, seven of eight cats immunized with OC-2 were positive to OC-2, six of eight cats immunized with OC-22 were positive to OC-22, and one of four cats immunized with Tg-50 were positive to Tg-50. Similar to the western blot analysis for the protein immunogenicity study described above, faint IgA responses from all of the OC-22 sero-positive animals could be observed. ELISA analysis of sera taken one week after the boost indicated that four out of eight cats immunized with OC-2 and four of eight cats immunized with OC-22 had midpoint titers greater than 1:1000.

The T cell analysis demonstrated positive proliferative responses to several antigens, however the data were difficult to interpret. Cells isolated from two cats immunized with the OC-22 gene and one cat immunized with the OC-2 gene each demonstrated significant SI responses. However, the same cells from each of these cats were also stimulated by the other recombinant antigen; i.e. cells from OC-22-injected cats responded to OC-2 protein and cells from OC-2-injected cats responded to OC-22 protein. Sera from these animals did not react with the poly histidine or *myc* fusions on other control fusion proteins. This inability to demonstrate strong proliferative responses in PBMC is consistent with other results observed while exploring the induction of proliferative responses in T cells from DNA immunized cats. Cat peripheral blood is a poor source of responsive T cells.

#### Analysis of Oocyst Shedding in Protein and DNA immunized cats:

Analysis of oocysts shed following tissue cyst challenge of cats in both the protein and DNA immunogenicity studies showed no significant difference in oocyst shedding between any of the test groups and the control within each study. However, the number of animals in these studies varied between two and four per group, and thus

this result is statistically meaningless. However, significant reduction, i.e., greater than several logs of total oocysts, was not observed in this experiment.

Example 18:

This example describes immunization of cats with nucleic acid molecules encoding immunogenic *T. gondii* proteins, and subsequent challenge of the immunized cats.

Immunization Protocol:

The following set of conditions were used for the delivery of DNA-coated gold particles to cats: 1.25 ug of DNA was delivered per shot by Gene Gun (available from Biorad). 1.6 micron gold particles were used in the presence of 0.05 mg/ml PVP (polyvinyl pyrrolidine, 360 kD). The micro-carrier loading quantity was 0.5 mg DNA/cartridge, while the DNA loading ratio was 2.5 ug DNA/mg gold. The animals were anesthetized and shaved at the points of contact with the gun. A total of six shots were delivered to the animal for each immunization: three shots to the inner thigh at 300 psi and three shots to the lower side of the abdomen at 600 psi. The immunization regimen consisted of one prime and two boosts at six week intervals. Tissue cyst challenge was performed two weeks following the second boost. The challenge was with 1000 mouse brain-derived tissue cysts.

The plasmid containing the human growth hormone (*hGH*) gene was used in the control groups and as a marker in the other groups in all studies. In most control groups, the *hGH* plasmid was diluted to a concentration similar to that in the test groups. Humoral immune responses to the gene product were measured with an ELISA assay, and cellular responses were measured using *hGH* protein.

First immunogenicity study: The first immunization was followed by a challenge of 1000 mouse brain derived tissue cysts fourteen weeks later. Sample collection was terminated three weeks after that. There were four groups of five animals per group, as follows: Group 1: Control, *hGH* (0.125 ug/shot), pDVacIII (1.125 ug/shot) This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 2: OC-22 in pDVacIII (1.25 ug/shot). This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 3: *hGH* (0.125 ug/shot), 9 *Toxoplasma* nucleic acid molecules OC-2, OC-22, OC-13, OC-14, Tg-41, Tg-45, Tg-50, 4604-3, and 4CQA11

(0.125 ug/shot). This group received one prime and two boosts, at 0, 6 and 12 weeks, respectively. Group 4: hGH (0.125 ug/shot), the same DNA as in Group 3 (9 *Toxoplasma* nucleic acid molecules), but this group received one prime and one boost, at 6 and 12 weeks, respectively. ELISA analysis for hGH sero conversion using sera  
5 collected throughout the study demonstrated that five of five cats in Group 1 were positive (i.e., demonstrated an end point titer > 1,000). Three of five animals in Group 3 were sero-positive to hGH. ELISA analysis for sero conversion to OC-22 protein using sera from Group 2 and Group 3 indicated that three of five and zero of five cats were positive respectively. These data suggest that competition from the other plasmids  
10 reduced the rate of sero conversion to an individual plasmid. In all cases positive titers did not occur until after the first boost. Specific-T cell proliferative responses using PBMC from animals in each group were not observed. Using the *B1* gene-based PCR ELISA herein described, the average number of oocysts shed for each group was: Group 1, 1.03e8; Group 2, 1.11e8; Group 3, 5.79e7 and Group 4, 8.83e7. Statistical  
15 analysis of the data indicated no significant difference between the test groups and the control.

Second immunogenicity study:

The first immunization for this study was followed by a challenge of 1000 mouse brain derived tissue cysts fourteen weeks later. Sample collection was terminated three  
20 weeks after that. There were four groups of five animals per group, and all animals received one prime and two boosts. Group 2 consisted of DNA representing 18 nucleic acid molecules of the present invention. Group 3 represent 14 additional nucleic acid molecules of the present invention. Group 4 was a combination of both of these groups. The specific nucleic acid molecules and concentrations used in the immunizations were  
25 as follows: Group 1: Control, *hGH* (0.083 ug/shot), pDVacIII (1.125 ug/shot). Group 2: *hGH* (0.070 ug/shot), 18 *Toxoplasma* nucleic acid molecules (BZ1-2, 4604-2, 4604-62, 4CQA27, 4CQA29, 4CQA21, 4CQA27, 4604-62, Q2-4, R8050-6, Tg50, M2A1, M2A5, M2A7, M2A11, M2A19, M2A22, M2A29) (0.070 ug/shot). Group 3: *hGH* (0.083 ug/shot), 14 *Toxoplasma* nucleic acid molecules (M2A3, M2A21, M2A18,  
30 M2A20, M2A24, M2A6, Q2-9, Q2-10, Q2-11, 4604-63, 4604-17, 4604-69, 4604-54,

4CQA19) (0.083 ug/shot). Group 4: *hGH* (0.040 ug/shot), 32 *Toxoplasma* nucleic acid molecules (BZ1-2, 4604-2, 4604-62, 4CQA27, 4CQA29, 4CQA21, 4CQA27, 4604-62, Q2-4, R8050-6, Tg-50, M2A1, M2A5, M2A7, M2A11, M2A19, M2A22, M2A29, M2A3, M2A21, M2A18, M2A20, M2A24, M2A6, Q2-9, Q2-10, Q2-11, 4604-63, 4604-17, 5 4604-69, 4604-54, 4CQA19) (0.040 ug/shot).

The ELISA analysis of antibody to *hGH* protein demonstrated that two of five, three of five, zero of five, and two of five animals seroconverted in Groups 1, 2, 3, and 4 respectively. Using low amounts of *hGH* plasmid in the presence of eighteen or thirty-two additional plasmids containing nucleic acid molecules of the present invention still 10 induced sero conversion in several animals per group. This observation suggests that there is not a strict reduction in the production of antibodies when a gene is injected with several other constructs.



What is claimed is:

1. An isolated nucleic acid molecule encoding an immunogenic *T. gondii* protein that can be identified by a method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum, wherein said antiserum is selected from the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection; and b) identifying a nucleic acid molecule in said library that expresses a protein that selectively binds to an antibody in said antiserum.
2. A method to isolate a nucleic acid molecule encoding an immunogenic *T. gondii* protein, said method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum selected the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum from a cat immune to *T. gondii* infection; b) identifying a nucleic acid molecule in said library that expresses a protein that selectively binds to an antibody in said antiserum; and c) recovering said nucleic acid molecule from said library.
3. An isolated immunogenic *T. gondii* protein that can be identified by a method comprising: a) immunoscreening a library selected from the group consisting of a *T. gondii* genomic expression library and a *T. gondii* cDNA expression library with an antiserum, wherein said antiserum is selected from the group consisting of antiserum raised against *T. gondii* oocysts, antiserum raised against *T. gondii* bradyzoites, antiserum raised against *T. gondii* infected cat gut, and antiserum isolated from a cat immune to *T. gondii* infection; and b) identifying a protein expressed from said library that selectively binds to antibodies in said antiserum.
4. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID

NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.

5. An isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid molecule selected from the group consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>, nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>, nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>, nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>, nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>, nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>, nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>, nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>, nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>, nTG78<sub>444</sub> and nTG79<sub>928</sub>.

6. An isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the

complement of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343.

7. An isolated immunogenic protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid molecule selected from the group consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>, nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>478</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>, nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>1106</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>, nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1231</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>, nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>, nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>440</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>, nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>, nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>666</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>, nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>, nTG78<sub>444</sub> and nTG79<sub>928</sub>.

8. A composition to inhibit *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, said composition comprising a compound selected from the group consisting of: an isolated immunogenic *T. gondii* protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising the
- 5 complement of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID
- 10 NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID
- 15 NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID
- 20 NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343; an isolated antibody that selectively binds to said immunogenic *T. gondii* protein; and an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a gene comprising a nucleic acid sequence selected from
- 25 the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID
- 30 NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID

NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID  
NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID  
NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID  
NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID  
5 NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID  
NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID  
NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID  
NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID  
NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID  
10 NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID  
NO:273, SEQ ID NO:340, and SEQ ID NO:343.

9. A method to inhibit *T. gondii* oocyst shedding in a cat due to infection with *T. gondii*, said method comprising administering to said cat a composition comprising a  
compound selected from the group consisting of: a) an isolated nucleic acid molecule  
15 that hybridizes under stringent hybridization conditions with a gene comprising a nucleic  
acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ  
ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID  
NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID  
NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID  
20 NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID  
NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID  
NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID  
NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID  
NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID  
25 NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID  
NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID  
NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID  
NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID  
NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID  
30 NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID

- NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343. b) an isolated immunogenic *T. gondii* protein encoded by a nucleic acid molecule that
- 5 hybridizes under stringent hybridization conditions with a gene comprising the complement of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID
- 10 NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID
- 15 NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID
- 20 NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343; and c) an isolated antibody that selectively binds to said immunogenic
- 25 *T. gondii* protein.

10. The invention of Claim 1, 2 or 3 wherein said antiserum isolated from a cat immune to *T. gondii* infection is enriched for antibodies to *T. gondii* gametogenic stages.

11. An isolated antibody that selectively binds to a protein as set forth in Claim 3, 6 or 7.

12. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID  
5 NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID  
10 NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID  
15 NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and  
20 SEQ ID NO:343.

13. The nucleic acid molecule of Claim 4 or 5, wherein said nucleic acid molecule encodes an immunogenic *T. gondii* protein.

14. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule comprises a nucleic acid sequence that is at least about 75% identical to a nucleic acid  
25 sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID  
30 NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID

NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID  
NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID  
NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID  
NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID  
5 NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID  
NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID  
NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID  
NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID  
NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID  
10 NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID  
NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343.

15. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule  
is selected from the group consisting of: a nucleic acid molecule comprising a nucleic  
acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ  
15 ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID  
NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID  
NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID  
NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID  
NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID  
20 NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID  
NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID  
NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID  
NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID  
NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID  
25 NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID  
NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID  
NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID  
NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID  
NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID  
30 NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID



NO:306, SEQ ID NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343.;  
and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule  
selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ  
ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID  
5 NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID  
NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID  
NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID  
NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID  
NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID  
10 NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID  
NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID  
NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID  
NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID  
NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID  
15 NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID  
NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID  
NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID  
NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID  
NO:271, SEQ ID NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID  
20 NO:311, SEQ ID NO:338, SEQ ID NO:340, and SEQ ID NO:343..

16. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule  
encodes a protein comprising an amino acid sequence that is at least about 75% identical  
to an amino acid sequence encoded by a nucleic acid molecule selected from the group  
consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID  
25 NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID  
NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID  
NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID  
NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID  
NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID  
30 NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID

NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID  
NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID  
NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID  
NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID  
5 NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID  
NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID  
NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID  
NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID  
NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID  
10 NO:273, SEQ ID NO:282, SEQ ID NO:292, SEQ ID NO:306, SEQ ID NO:311, SEQ ID  
NO:338, SEQ ID NO:340, and SEQ ID NO:343.

17. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule  
hybridizes under stringent hybridization conditions with a nucleic acid molecule  
comprising a nucleic acid sequence encoding a protein comprising an amino acid  
15 sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID  
NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16,  
SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ  
ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID  
NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID  
20 NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID  
NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID  
NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID  
NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID  
NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID  
25 NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID  
NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID  
NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID  
NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID  
NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID  
30 NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and  
SEQ ID NO:341.

18. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said amino acid sequences.

19. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule comprises an oligonucleotide.

20. A recombinant molecule comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5, operatively linked to a transcription control sequence.

21. A recombinant virus comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5.

22. A recombinant cell comprising a nucleic acid molecule as set forth in Claim 1, 4 or 5.

23. The nucleic acid molecule of Claim 5, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule selected from the group consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>, nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>,  
 5 nTG17<sub>690</sub>, nTG18<sub>313</sub>, nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>, nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>, nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>, nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>, nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>,  
 10 nTG61<sub>390</sub>, nTG62<sub>699</sub>, nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>, nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>, nTG78<sub>444</sub>, nTG79<sub>928</sub>, nTG22<sub>310a</sub>, nTG64<sub>303a</sub>, nTG71<sub>236a</sub>, nTG6<sub>425a</sub>, nTG41<sub>513a</sub>, nTG<sub>867</sub>, nTG<sub>1397</sub>, nTG<sub>1785</sub>.

24. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule  
 15 is at least about 75% identical to a nucleic acid molecule selected from the group consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>, nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>, nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>, nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>,  
 20 nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>, nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>, nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>, nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>, nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>,  
 25 nTG78<sub>444</sub>, nTG79<sub>928</sub>, nTG22<sub>310a</sub>, nTG64<sub>303a</sub>, nTG71<sub>236a</sub>, nTG6<sub>425a</sub>, nTG41<sub>513a</sub>, nTG<sub>867</sub>, nTG<sub>1397</sub>, nTG<sub>1785</sub>.

25. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule selected from the group consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>,  
 30 nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>,

- nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>,  
nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>,  
nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>,  
nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>,  
5 nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>,  
nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>,  
nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>,  
nTG78<sub>444</sub>, nTG79<sub>928</sub>, nTG22<sub>310a</sub>, nTG64<sub>303a</sub>, nTG71<sub>236a</sub>, nTG6<sub>425a</sub>, nTG41<sub>513a</sub>, nTG<sub>867</sub>,  
nTG<sub>1397</sub>, nTG<sub>1785</sub>; and a nucleic acid molecule comprising an allelic variant of a nucleic  
10 acid molecule selected from the group consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>,  
nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>, nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>,  
nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>, nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>,  
nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>, nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>,  
nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>, nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>,  
15 nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>, nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>,  
nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>, nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>,  
nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>, nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>,  
nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>, nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>,  
nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>, nTG78<sub>444</sub>, nTG79<sub>928</sub>, nTG22<sub>310a</sub>, nTG64<sub>303a</sub>,  
20 nTG71<sub>236a</sub>, nTG6<sub>425a</sub>, nTG41<sub>513a</sub>, nTG<sub>867</sub>, nTG<sub>1397</sub>, nTG<sub>1785</sub>.
26. The nucleic acid molecule of Claim 1, 4 or 5, wherein said nucleic acid molecule  
encodes a protein comprising an amino acid sequence that is at least about 75% identical  
to an amino acid sequence encoded by a nucleic acid molecule selected from the group  
consisting of nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>,  
25 nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>,  
nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>,  
nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>, nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>,  
nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>,  
nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>,  
30 nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>,

nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>,  
 nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>, nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>,  
 nTG78<sub>444</sub>, nTG79<sub>928</sub>, nTG22<sub>310a</sub>, nTG64<sub>303a</sub>, nTG71<sub>236a</sub>, nTG6<sub>425a</sub>, nTG41<sub>513a</sub>, nTG<sub>867</sub>,  
 nTG<sub>1397</sub>, nTG<sub>1785</sub>.

- 5 27. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: a protein comprising an amino acid sequence that is at least about 75% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and SEQ ID NO:341; and a protein comprising an epitope of said protein having said amino acid sequence.
- 25 28. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein encoded by a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID
- 30

NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:50, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:340, and SEQ ID NO:343; and an immunogenic protein encoded by a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

29. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected from the group consisting of: an immunogenic protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID

NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:140, SEQ ID NO:142, SEQ ID NO:144, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:283, SEQ ID NO:293, SEQ ID NO:307, SEQ ID NO:312, and

5 SEQ ID NO:341.; and an immunogenic *T. gondii* protein encoded by an allelic variant of a nucleic acid molecule encoding a protein comprising any of said amino acid sequences.

30. The immunogenic protein of Claim 3, 6 or 7, wherein said protein comprises an epitope that elicits an immune response.

31. The immunogenic protein of Claim 3, 6 or 7, wherein said protein is selected  
10 from the group consisting of: an immunogenic protein encoded by a nucleic acid molecule selected from the group consisting of: nTG2<sub>339</sub>, nTG4<sub>526</sub>, nTG4<sub>1478</sub>, nTG5<sub>657</sub>, nTG5<sub>1029</sub>, nTG6<sub>425</sub>, nTG7<sub>417</sub>, nTG8<sub>507</sub>, nTG9<sub>718</sub>, nTG10<sub>441</sub>, nTG11<sub>428</sub>, nTG13<sub>282</sub>, nTG15<sub>304</sub>, nTG16<sub>284</sub>, nTG17<sub>690</sub>, nTG18<sub>313</sub>, nTG19<sub>389</sub>, nTG21<sub>548</sub>, nTG22<sub>310</sub>, nTG23<sub>220</sub>, nTG24<sub>642</sub>, nTG25<sub>381</sub>, nTG26<sub>432</sub>, nTG27<sub>282</sub>, nTG28<sub>466</sub>, nTG30<sub>539</sub>, nTG31<sub>1233</sub>, nTG32<sub>411</sub>, nTG33<sub>441</sub>,  
15 nTG34<sub>491</sub>, nTG35<sub>387</sub>, nTG36<sub>417</sub>, nTG37<sub>416</sub>, nTG38<sub>500</sub>, nTG40<sub>321</sub>, nTG41<sub>513</sub>, nTG42<sub>528</sub>, nTG43<sub>375</sub>, nTG44<sub>543</sub>, nTG45<sub>573</sub>, nTG46<sub>1835</sub>, nTG48<sub>604</sub>, nTG48<sub>549</sub>, nTG49<sub>270</sub>, nTG50<sub>306</sub>, nTG51<sub>804</sub>, nTG52<sub>867</sub>, nTG53<sub>1434</sub>, nTG54<sub>680</sub>, nTG55<sub>296</sub>, nTG56<sub>723</sub>, nTG57<sub>270</sub>, nTG58<sub>503</sub>, nTG60<sub>322</sub>, nTG61<sub>390</sub>, nTG62<sub>699</sub>, nTG63<sub>419</sub>, nTG64<sub>303</sub>, nTG65<sub>696</sub>, nTG66<sub>173</sub>, nTG67<sub>369</sub>, nTG68<sub>566</sub>, nTG69<sub>616</sub>, nTG70<sub>762</sub>, nTG71<sub>236</sub>, nTG72<sub>569</sub>, nTG73<sub>232</sub>, nTG74<sub>276</sub>, nTG75<sub>309</sub>,  
20 nTG76<sub>534</sub>, nTG76<sub>423</sub>, nTG77<sub>327</sub>, nTG78<sub>444</sub>, nTG79<sub>928</sub>, nTG22<sub>310a</sub>, nTG64<sub>303a</sub>, nTG71<sub>236a</sub>, nTG6<sub>425a</sub>, nTG41<sub>513a</sub>, nTG<sub>867</sub>, nTG<sub>1397</sub>, nTG<sub>1785</sub>; and an immunogenic protein encoded by a nucleic acid molecule comprising an allelic variant of any of said nucleic acid molecules.

32. The invention of Claim 8 or 9, wherein said composition further comprises a  
25 component selected from the group consisting of an excipient, an adjuvant, and a carrier.

33. The invention of Claim 8 or 9, wherein said compound is selected from the group consisting of a genetic vaccine, a recombinant virus vaccine and a recombinant cell vaccine.

34. The invention of Claim 8 or 9, wherein said compound comprises a recombinant  
30 molecule.



35. The invention of Claim 8 or 9, wherein said compound is selected from the group consisting of a recombinant virus genome and a recombinant plasmid.

36. The invention of Claim 8 or 9, wherein said composition is administered by a method selected from the group consisting of injection, oral administration, inhalation,  
5 nasal administration, intraocular administration, anal administration, topical administration, particle bombardment, and intradermal scarification.

37. The invention of Claim 8 or 9, wherein said composition is administered by a method selected from the group consisting of intradermal injection and intramuscular injection.

10 38. The invention of Claim 8 or 9, wherein said composition is administered mucosally.

39. A method to detect parasite cysts or oocysts in feces, said method comprising:

1. contacting a sample of feces with a solid support capable of binding oocysts;
2. allowing the sample to dry onto the solid support;
- 15 3. washing the sample on the solid support with an aqueous wash solution;
4. adding an aqueous elution solution to the sample and eluting DNA from the sample into the aqueous elution solution by heating;
5. PCR amplifying oocyst-specific DNA with primers specific to the oocyst being detected; and
- 20 6. detecting the presence of a PCR amplification product resulting from amplification of oocyst-specific DNA in the sample wherein the presence of said product indicates the presence of cysts or oocysts in said feces.

40. A method according to Claim 39, wherein the sample of feces is solubilized in an aqueous solution before contacting the sample with a solid support capable of binding  
25 oocysts.

41. A method according to Claim 39, wherein the aqueous wash solution comprises distilled water.

42. A method according to Claim 39, wherein the aqueous elution solution comprises distilled water.

-156-

43. A method according to Claim 39, wherein the heating step comprises heating to approximately 95° C.
44. A method according to Claim 39, wherein the solid support capable of binding oocysts comprises paper.
- 5 45. A method according to Claim 39, wherein the solid support comprises one or more compounds capable of binding inhibitors of PCR amplification.
46. The method of Claim 39, wherein the parasite oocysts are enteric apicomplexa oocysts.
47. The method of Claim 46 wherein the enteric apicomplexa oocysts are selected
- 10 from the group consisting of *Cryptosporidium* oocysts and *Toxoplasma* oocysts.

## SEQUENCE LISTING

<110> Milhausen, Michael James  
Lutz, Susan Bektesh  
Ng, Ray K.

<120> TOXOPLASMA GONDII PROTEINS, NUCLEIC ACID MOLECULES, AND  
USES THEREOF

<130> TX-1-C1-PCT

<140> 08/994,825

<141> 1997-12-19

<150> 08/994,825

<151> 1997-12-19

<160> 364

<170> PatentIn Ver. 2.0

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<213> Toxoplasma gondii

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tggttt cca agc ggc tct gtt ctt ctc tct tct tcg ccg gcg cat tcc	96
Trp Ile Pro Ser Gly Ser Val Leu Leu Ser Ser Ser Pro Ala His Ser	
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Ala Gly Pro Arg Asn Thr Arg Thr Ser Arg Val Ala Arg Val Gly Leu	
35 40 45	
gaa gcc ggg cct gcg aaa ggc gag aca gaa agc aga cga agc aga cag	192
Glu Ala Gly Pro Ala Lys Gly Glu Thr Glu Ser Arg Arg Ser Arg Gln	
50 55 60	
gac gaa ggg agc gac gtc cgt ggg cgc ttt ttt cga ggc aga caa acc	240

Asp Glu Gly Ser Asp Val Arg Gly Arg Phe Phe Arg Gly Arg Gln Thr  
 65 70 75 80  
  
 gga gac tct cgc atc cac atg ggg gtc tac gag ggt cac gat ggc aac 288  
 Gly Asp Ser Arg Ile His Met Gly Val Tyr Glu Gly His Asp Gly Asn  
 85 90 95  
  
 gag ttc ggc gaa gag aga gaa caa ggt gca tgc gat ttt tcc gca tct 336  
 Glu Phe Gly Glu Glu Arg Glu Gln Gly Ala Cys Asp Phe Ser Ala Ser  
 100 105 110  
  
 cct cgc tcg gaa ggc gga tcg 357  
 Pro Arg Ser Glu Gly Gly Ser  
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 Ala Gly Pro Arg Asn Thr Arg Thr Ser Arg Val Ala Arg Val Gly Leu  
 35 40 45  
  
 Glu Ala Gly Pro Ala Lys Gly Glu Thr Glu Ser Arg Arg Ser Arg Gln  
 50 55 60  
  
 Asp Glu Gly Ser Asp Val Arg Gly Arg Phe Phe Arg Gly Arg Gln Thr  
 65 70 75 80  
  
 Gly Asp Ser Arg Ile His Met Gly Val Tyr Glu Gly His Asp Gly Asn  
 85 90 95  
  
 Glu Phe Gly Glu Glu Arg Glu Gln Gly Ala Cys Asp Phe Ser Ala Ser  
 100 105 110  
  
 Pro Arg Ser Glu Gly Gly Ser  
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ggg ggc gcg aca gcc gcc gaa aca gct gct gcg cga ata gct gct ctc 96  
 Gly Gly Ala Thr Ala Ala Glu Thr Ala Ala Ala Arg Ile Ala Ala Leu  
 20 25 30

aaa cca tcg ctc ttc gcg gcc tcc gaa ctc cct ccc gat gag aca gca 144  
 Lys Pro Ser Leu Phe Ala Ala Ser Glu Leu Pro Pro Asp Glu Thr Ala  
 35 40 45

gac agt gga gac aca ggg ccg ttc cgg cga gac cgg gac ttc ttc gcc 192  
 Asp Ser Gly Asp Thr Gly Pro Phe Arg Arg Asp Arg Asp Phe Phe Ala  
 50 55 60

ggc acc gcg ggc gaa cac gat gcg tcg gcg atg cga gac aaa gag gcc 240  
 Gly Thr Ala Gly Glu His Asp Ala Ser Ala Met Arg Asp Lys Glu Ala  
 65 70 75 80

gca ggc tcc tca ggc gaa gag aca ccc gca gag atc ggc atc ttg ggc 288  
 Ala Gly Ser Ser Gly Glu Glu Thr Pro Ala Glu Ile Gly Ile Leu Gly  
 85 90 95

agc cgc gga aac agt tgg ccg gga gac caa ggc tgc tgaagcgctcg ctcgc 339  
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 100 105

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 35 40 45  
 Asp Ser Gly Asp Thr Gly Pro Phe Arg Arg Asp Arg Asp Phe Phe Ala  
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 Gly Thr Ala Gly Glu His Asp Ala Ser Ala Met Arg Asp Lys Glu Ala  
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 Ala Gly Ser Ser Gly Glu Glu Thr Pro Ala Glu Ile Gly Ile Leu Gly  
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 100 105

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 tcc tct ctt ccg cac ttt cca tct gtg ttt cca gcg agc gcc gta gtc 96  
 Ser Ser Leu Pro His Phe Pro Ser Val Phe Pro Ala Ser Ala Val Val  
 20 25 30  
 tcc ccc atc gac gaa aac cct gca gag atg gaa agc acc atc tcc gag 144  
 Ser Pro Ile Asp Glu Asn Pro Ala Glu Met Glu Ser Thr Ile Ser Glu  
 35 40 45  
 ggc gaa gca ggt tct gcg gtg gcg gct cct gaa caa ggt atc cag cca 192  
 Gly Glu Ala Gly Ser Ala Val Ala Ala Pro Glu Gln Gly Ile Gln Pro  
 50 55 60  
 gag gca gaa ttt gct acc gcc agc gaa gaa cca cgt ccc ctg gaa cct 240  
 Glu Ala Glu Phe Ala Thr Ala Ser Glu Glu Pro Arg Pro Leu Glu Pro  
 65 70 75 80

gtc gac ccc gaa atg gca gct cag cag ccg caa ctg cct' caa gaa gct 288  
 Val Asp Pro Glu Met Ala Ala Gln Gln Pro Gln Leu Pro Gln Glu Ala  
                     85                                    90                                    95

atg cca act gag aat gcg gac ctt ctt gga aac cag ccc aga atg cgc 336  
 Met Pro Thr Glu Asn Ala Asp Leu Leu Gly Asn Gln Pro Arg Met Arg  
                     100                                    105                                    110

aat gct ctc gaa ccc tct gcc aag gtc ctc gaa ccg gaa acc ctg gaa 384  
 Asn Ala Leu Glu Pro Ser Ala Lys Val Leu Glu Pro Glu Thr Leu Glu  
                     115                                    120                                    125

ggg tca cct gct ctc gtc ccg ccg gca gag act gaa gag ggg aca gcc 432  
 Gly Ser Pro Ala Leu Val Pro Pro Ala Glu Thr Glu Glu Gly Thr Ala  
                     130                                    135                                    140

gcc caa att gcg gag gaa atg agc aag cag gat cag ggc atg cag gaa 480  
 Ala Gln Ile Ala Glu Glu Met Ser Lys Gln Asp Gln Gly Met Gln Glu  
                     145                                    150                                    155                                    160

gcc agg cct caa gaa gtt ctc agt aag caa tgg gtt ctt cga tat t 526  
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<213> Toxoplasma gondii

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                     20                                    25                                    30

Ser Pro Ile Asp Glu Asn Pro Ala Glu Met Glu Ser Thr Ile Ser Glu  
                     35                                    40                                    45

Gly Glu Ala Gly Ser Ala Val Ala Ala Pro Glu Gln Gly Ile Gln Pro  
                     50                                    55                                    60

Glu Ala Glu Phe Ala Thr Ala Ser Glu Glu Pro Arg Pro Leu Glu Pro  
                     65                                    70                                    75                                    80

Val Asp Pro Glu Met Ala Ala Gln Gln Pro Gln Leu Pro Gln Glu Ala  
                     85                                    90                                    95

Met Pro Thr Glu Asn Ala Asp Leu Leu Gly Asn Gln Pro Arg Met Arg  
 100 105 110

Asn Ala Leu Glu Pro Ser Ala Lys Val Leu Glu Pro Glu Thr Leu Glu  
 115 120 125

Gly Ser Pro Ala Leu Val Pro Pro Ala Glu Thr Glu Glu Gly Thr Ala  
 130 135 140

Ala Gln Ile Ala Glu Glu Met Ser Lys Gln Asp Gln Gly Met Gln Glu  
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<213> *Toxoplasma gondii*

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gaa gcc ggt ttg ttc cgc aag gct gtt ccg ggc ttg gac gac cca gct 99  
 Glu Ala Gly Leu Phe Arg Lys Ala Val Pro Gly Leu Asp Asp Pro Ala  
 15 20 25

gaa gac gat gaa gcc gac ggc gaa agt gcc tcg gat gag gca gaa gcc 147  
 Glu Asp Asp Glu Ala Asp Gly Glu Ser Ala Ser Asp Glu Ala Glu Ala  
 30 35 40

gac tct gac gtc ttg gcg gac gac gaa gaa ggg aca tcc ctc atc gaa 195  
 Asp Ser Asp Val Leu Ala Asp Asp Glu Glu Gly Thr Ser Leu Ile Glu  
 45 50 55

aat gcg agt gag gaa gac aca gac aac tcc gag gca gac agt caa cag 243  
 Asn Ala Ser Glu Glu Asp Thr Asp Asn Ser Glu Ala Asp Ser Gln Gln  
 60 65 70 75

gag gat gac agt gtg gga gag gat tcc ttt ctc cag cag gag ggc gag 291



Glu Asp Asp Ser Val Gly Glu Asp Ser Phe Leu Gln Gln Glu Gly Glu	
80 85 90	
gac tcc gag gaa gaa aga gca gtc gag gac ccg tat gct gcc gcc gaa	339
Asp Ser Glu Glu Glu Arg Ala Val Glu Asp Pro Tyr Ala Ala Ala Glu	
95 100 105	
ccc tct tat ctt gaa gag gac aac act gtt gac gac agc gcg gcg gag	387
Pro Ser Tyr Leu Glu Glu Asp Asn Thr Val Asp Asp Ser Ala Ala Glu	
110 115 120	
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Asp Tyr Ala Pro Ala Ser Phe Val Gln Ile Gly Ser Gly Glu Arg Lys	
125 130 135	
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Phe Ala His Ala Phe Asn Gln Asp His Val Arg Leu Leu Asp Gln Thr	
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gcc gtc gag gac gaa ctt ctc gat gag gcc gcc ccg ggc gga ggc gcg	579
Ala Val Glu Asp Glu Leu Leu Asp Glu Ala Ala Pro Gly Gly Gly Ala	
175 180 185	
agc gcc gta gtc tcc ccc atc gac gaa aac cct gca gag atg gaa agc	627
Ser Ala Val Val Ser Pro Ile Asp Glu Asn Pro Ala Glu Met Glu Ser	
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acc atc tcc gag ggc gaa gca ggt tct gcg gtg gcg gct cct gaa caa	675
Thr Ile Ser Glu Gly Glu Ala Gly Ser Ala Val Ala Ala Pro Glu Gln	
205 210 215	
ggt atc cag cca gag gca gaa ttt gct acc gcc agc gaa gaa cca cgt	723
Gly Ile Gln Pro Glu Ala Glu Phe Ala Thr Ala Ser Glu Glu Pro Arg	
220 225 230 235	
ccc ctg gaa cct gtc gac ccc gaa atg gca gct cag cag ccg caa ctg	771
Pro Leu Glu Pro Val Asp Pro Glu Met Ala Ala Gln Gln Pro Gln Leu	
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cct caa gaa gct atg cca act gag aat gcg gac ctt ctt gga aac cag	819
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255 260 265	
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Pro Arg Met Arg Asn Ala Leu Glu Pro Ser Ala Lys Val Leu Glu Pro  
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 285 290 295

gag ggg aca gcc gcc caa att gcg gag gaa atg agc aag cag gat cag 963  
 Glu Gly Thr Ala Ala Gln Ile Ala Glu Glu Met Ser Lys Gln Asp Gln  
 300 305 310 315

ggc atg cag gaa gcc agg cct caa gaa gtt ctc aca cga cac acc tgg 1011  
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 320 325 330

caa gat atg gag aga act gag gac cta cga aag aac gac gtc ccg gct 1059  
 Gln Asp Met Glu Arg Thr Glu Asp Leu Arg Lys Asn Asp Val Pro Ala  
 335 340 345

gca gtg gcg aat tcc ggc agc cag atc atc acg gct gcg tcg tcc gtc 1107  
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 350 355 360

gcc ctt gct ggt cta ctg gtc gca gga cag ctt ttg ttc agc gtg ggc 1155  
 Ala Leu Ala Gly Leu Leu Val Ala Gly Gln Leu Leu Phe Ser Val Gly  
 365 370 375

atg tac tgagaataat attcttgctt cgtggaatat tgttgctacc tgaaagttaa 1211  
 Met Tyr  
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aagctaactc ttttccggca taacttcgtt tttggtgtca aaaaaaaaaa aaaaaaaaaa 1451

aaaaaaaaaa aaaaaaaaaa aaaaaaa 1478

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&lt;211&gt; 381

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 8

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 35 40 45  
 Ala Asp Asp Glu Glu Gly Thr Ser Leu Ile Glu Asn Ala Ser Glu Glu  
 50 55 60  
 Asp Thr Asp Asn Ser Glu Ala Asp Ser Gln Gln Glu Asp Asp Ser Val  
 65 70 75 80  
 Gly Glu Asp Ser Phe Leu Gln Gln Glu Gly Glu Asp Ser Glu Glu Glu  
 85 90 95  
 Arg Ala Val Glu Asp Pro Tyr Ala Ala Ala Glu Pro Ser Tyr Leu Glu  
 100 105 110  
 Glu Asp Asn Thr Val Asp Asp Ser Ala Ala Glu Asp Tyr Ala Pro Ala  
 115 120 125  
 Ser Phe Val Gln Ile Gly Ser Gly Glu Arg Lys Ile Arg Ala His Met  
 130 135 140  
 His Leu Asp Ser Arg Gln Val Ala Pro Glu Arg Phe Ala His Ala Phe  
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 Asn Gln Asp His Val Arg Leu Leu Asp Gln Thr Ala Val Glu Asp Glu  
 165 170 175  
 Leu Leu Asp Glu Ala Ala Pro Gly Gly Gly Ala Ser Ala Val Val Ser  
 180 185 190  
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 195 200 205  
 Glu Ala Gly Ser Ala Val Ala Ala Pro Glu Gln Gly Ile Gln Pro Glu  
 210 215 220  
 Ala Glu Phe Ala Thr Ala Ser Glu Glu Pro Arg Pro Leu Glu Pro Val  
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Pro Thr Glu Asn Ala Asp Leu Leu Gly Asn Gln Pro Arg Met Arg Asn  
 260 265 270

Ala Leu Glu Pro Ser Ala Lys Val Leu Glu Pro Glu Thr Leu Glu Gly  
 275 280 285

Ser Pro Ala Leu Val Pro Pro Ala Glu Thr Glu Glu Gly Thr Ala Ala  
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Gln Ile Ala Glu Glu Met Ser Lys Gln Asp Gln Gly Met Gln Glu Ala  
 305 310 315 320

Arg Pro Gln Glu Val Leu Thr Arg His Thr Trp Gln Asp Met Glu Arg  
 325 330 335

Thr Glu Asp Leu Arg Lys Asn Asp Val Pro Ala Ala Val Ala Asn Ser  
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Gly Ser Gln Ile Ile Thr Ala Ala Ser Ser Val Ala Leu Ala Gly Leu  
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<213> *Toxoplasma gondii*

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cca ttt gct gag ctg cca cag ctg gca gaa ata cca aag ctc tcc gaa 96  
 Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu  
 20 25 30

ctt ccg aaa atc gcg gac atg ccg aaa ttt tcg gat atg ccc aag atg 144  
 Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met  
 35 40 45

gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg ccc 192

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Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp															
65					70				75					80	
ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag atg															288
Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met															
				85				90						95	
gct gag atg cca aag tta tca gat atg ccc aga atg gct gac att cca															336
Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro															
			100				105						110		
cag ttt cca gag atg cct agg atg gtt gac atg cct cag ttt cca gaa															384
Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu															
		115					120						125		
atc ccc agg atg gct gat atg cgg aga ttt ccg gag atg tcc aag ata															432
Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile															
130						135							140		
gct gac atg cca aag ttt cca gac atg cca aac gtc act gag atg cca															480
Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro															
145					150								155		160
aag ctt gca gat ttg cca agg ctg gct gac atg ccc agt att gcc gac															528
Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp															
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Met Pro Arg Leu Ser Asp Met Pro Ser Ile Ala Asp Met Pro Arg Leu															
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Ser Asp Ile Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Met Pro															
		195						200					205		
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Ser Ile Ala Asp Met Pro Lys Phe Ser Ser Arg															
210						215									

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Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu  
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Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met  
 35 40 45

Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro  
 50 55 60

Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp  
 65 70 75 80

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met  
 85 90 95

Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro  
 100 105 110

Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu  
 115 120 125

Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile  
 130 135 140

Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro  
 145 150 155 160

Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp  
 165 170 175

Met Pro Arg Leu Ser Asp Met Pro Ser Ile Ala Asp Met Pro Arg Leu  
 180 185 190

Ser Asp Ile Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Met Pro  
 195 200 205

Ser Ile Ala Asp Met Pro Lys Phe Ser Ser Arg  
 210 215

&lt;210&gt; 11

&lt;211&gt; 1029

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(819)

&lt;400&gt; 11

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gag atg agc gcc cca gat agg caa aca gga aag ctt tcc gat tta ccg      48
Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro
  1              5              10              15

cca ttt gct gag ctg cca cag ctg gca gaa ata cca aag ctc tcc gaa      96
Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu
          20              25              30

ctt ccg aaa atc gcg gac atg ccg aaa ttt tcg gat atg ccc aag atg      144
Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met
          35              40              45

gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg ccc      192
Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro
          50              55              60

aag tta tca gat ata ccc aag atg gct gag atg ccc aag tta tca gat      240
Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp
          65              70              75              80

ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag atg      288
Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met
          85              90              95

gct gag atg cca aag tta tca gat atg ccc aga atg gct gac att cca      336
Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro
          100             105             110

cag ttt cca gag atg cct agg atg gtt gac atg cct cag ttt cca gaa      384
Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu
          115             120             125

atc ccc agg atg gct gat atg cgg aga ttt ccg gag atg tcc aag ata      432
Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile
          130             135             140

gct gac atg cca aag ttt cca gac atg cca aac gtc act gag atg cca      480
Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro
          145             150             155             160

aag ctt gca gat ttg cca agg ctt gct gac atg ccc agt att gcc gac      528

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Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp  
 165 170 175  
 atg ccc cgg ctg tca gac atg ccc agt att gca gac atg ccc cgg ctg 576  
 Met Pro Arg Leu Ser Asp Met Pro Ser Ile Ala Asp Met Pro Arg Leu  
 180 185 190  
 tca gac ata ccc agt att gcc gac atg ccc cgg ctg tca gac atg ccc 624  
 Ser Asp Ile Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Met Pro  
 195 200 205  
 agt att gcc gac atg ccg aaa ttc tct agt aac cga gtt cat ggg caa 672  
 Ser Ile Ala Asp Met Pro Lys Phe Ser Ser Asn Arg Val His Gly Gln  
 210 215 220  
 agt tac cat att ctg gcg ata tgg aca ccg tcc ctt tcc gga ctg aag 720  
 Ser Tyr His Ile Leu Ala Ile Trp Thr Pro Ser Leu Ser Gly Leu Lys  
 225 230 235 240  
 gag ttt ttt acc ccg ctg tct gac cta atc aag cca gaa gct gct tcc 768  
 Glu Phe Phe Thr Pro Leu Ser Asp Leu Ile Lys Pro Glu Ala Ala Ser  
 245 250 255  
 ctg aca agc ctg gcc aag cca tct gga gtt ttt ctg aga acc ctg ctg 816  
 Leu Thr Ser Leu Ala Lys Pro Ser Gly Val Phe Leu Arg Thr Leu Leu  
 260 265 270  
 gct tgatgagaaa atgtatattg acaaatggct gtatctccat agttatagtg 869  
 Ala  
 aggaatgtat tgacttattc cgaggactct atactgaacc cgcggcatac gaggaaactg 929  
 acaagttgggt gatgtgcgtt tctgatcttc cccgaaaaga aaaaaaaatg accgtcttaa 989  
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1029

&lt;210&gt; 12

&lt;211&gt; 273

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 12

Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro  
 1 5 10 15

Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu  
 20 25 30



Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met  
 35 40 45  
 Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro  
 50 55 60  
 Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp  
 65 70 75 80  
 Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met  
 85 90 95  
 Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro  
 100 105 110  
 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu  
 115 120 125  
 Ile Pro Arg Met Ala Asp Met Arg Arg Phe Pro Glu Met Ser Lys Ile  
 130 135 140  
 Ala Asp Met Pro Lys Phe Pro Asp Met Pro Asn Val Thr Glu Met Pro  
 145 150 155 160  
 Lys Leu Ala Asp Leu Pro Arg Leu Ala Asp Met Pro Ser Ile Ala Asp  
 165 170 175  
 Met Pro Arg Leu Ser Asp Met Pro Ser Ile Ala Asp Met Pro Arg Leu  
 180 185 190  
 Ser Asp Ile Pro Ser Ile Ala Asp Met Pro Arg Leu Ser Asp Met Pro  
 195 200 205  
 Ser Ile Ala Asp Met Pro Lys Phe Ser Ser Asn Arg Val His Gly Gln  
 210 215 220  
 Ser Tyr His Ile Leu Ala Ile Trp Thr Pro Ser Leu Ser Gly Leu Lys  
 225 230 235 240  
 Glu Phe Phe Thr Pro Leu Ser Asp Leu Ile Lys Pro Glu Ala Ala Ser  
 245 250 255  
 Leu Thr Ser Leu Ala Lys Pro Ser Gly Val Phe Leu Arg Thr Leu Leu  
 260 265 270  
 Ala

<210> 13  
 <211> 425  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <221> CDS  
 <222> (1)..(423)

<400> 13  
 cgc gga att ccg gat cag cgt agc agt cgc agc cac act gga gtg gaa 48  
 Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu  
     1                    5                    10                    15

agt ctg gtt ttg ccc tcc aga ggg gag gaa gag gcg aga gag gag acg 96  
 Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr  
                     20                    25                    30

tct gca acg cgc cag atg ccg acg ctt ctc tct tcg ccg agg cct cca 144  
 Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro  
             35                    40                    45

ctc gcg ctg ggg ttg gga gac aag tct ccc tgc gga gag tgg gtg tcg 192  
 Leu Ala Leu Gly Leu Gly Asp Lys Ser Pro Cys Gly Glu Trp Val Ser  
     50                    55                    60

ccg aat gac atg gtt tct gcg ttg tcc ctc tgg gaa gca ggc gag gct 240  
 Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala  
     65                    70                    75                    80

tgg cag ttc aag aca gcg aaa att ctt gac tct ttc gaa ggg gag acc 288  
 Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr  
                     85                    90                    95

cca gaa ggg gag gga tgc ggc gca cag gaa aga agg aca gcc gca tgc 336  
 Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Arg Arg Thr Ala Ala Cys  
             100                    105                    110

aag ctg gtg cga ctc ccg gtg aac gtg gag ggg cgg tcg aca aag gtg 384  
 Lys Leu Val Arg Leu Pro Val Asn Val Glu Gly Arg Ser Thr Lys Val  
     115                    120                    125

tgg agc ttg gct ctt ctt tct tct ctg cgt ctg aag atc cg 425  
 Trp Ser Leu Ala Leu Leu Ser Ser Leu Arg Leu Lys Ile  
     130                    135                    140

<210> 14  
 <211> 141  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 14  
 Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu  
 1 5 10 15  
 Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr  
 20 25 30  
 Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro  
 35 40 45  
 Leu Ala Leu Gly Leu Gly Asp Lys Ser Pro Cys Gly Glu Trp Val Ser  
 50 55 60  
 Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala  
 65 70 75 80  
 Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr  
 85 90 95  
 Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Arg Arg Thr Ala Ala Cys  
 100 105 110  
 Lys Leu Val Arg Leu Pro Val Asn Val Glu Gly Arg Ser Thr Lys Val  
 115 120 125  
 Trp Ser Leu Ala Leu Leu Ser Ser Leu Arg Leu Lys Ile  
 130 135 140

<210> 15  
 <211> 417  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(417)

<400> 15  
 cgc ggc ctt tcc gat gac gcc tct cac gcg gag acc cct tca ccg ctc 48  
 Arg Gly Leu Ser Asp Asp Ala Ser His Ala Glu Thr Pro Ser Pro Leu  
 1 5 10 15

acg ccc tcg agg gtg gac agc ttc tca gac gga gtt gag aga aca cgc 96  
 Thr Pro Ser Arg Val Asp Ser Phe Ser Asp Gly Val Glu Arg Thr Arg  
                   20                  25                  30

aga agc tct ccg cga gtc gag gag cac cag acg agc tcg aga gag gaa 144  
 Arg Ser Ser Pro Arg Val Glu Glu His Gln Thr Ser Ser Arg Glu Glu  
                   35                  40                  45

aaa gct gcg aca gag cgc gtt cca aaa ctg tct cgt ctc ccc tcg ctc 192  
 Lys Ala Ala Thr Glu Arg Val Pro Lys Leu Ser Arg Leu Pro Ser Leu  
                   50                  55                  60

cga gct cct cta cgc agc acg gac cga cgc gcc tcg ccg cct cgt cgg 240  
 Arg Ala Pro Leu Arg Ser Thr Asp Arg Arg Ala Ser Pro Pro Arg Arg  
                   65                  70                  75                  80

ctg tcg caa ctt ctt cgc tgc tgc aca acc tcg aga ttc gcg agc aaa 288  
 Leu Ser Gln Leu Leu Arg Cys Cys Thr Thr Ser Arg Phe Ala Ser Lys  
                   85                  90                  95

gga acg gcg tat cca gac gag gag tgg ggg cat aga gtc cga gca cag 336  
 Gly Thr Ala Tyr Pro Asp Glu Glu Trp Gly His Arg Val Arg Ala Gln  
                   100                  105                  110

aga aca gaa gag act gtc tcc tct ctg acg acg aag cgc ctt ctc act 384  
 Arg Thr Glu Glu Thr Val Ser Ser Leu Thr Thr Lys Arg Leu Leu Thr  
                   115                  120                  125

cga agt cct aat tcg cag act gcc ttc ccg cgg 417  
 Arg Ser Pro Asn Ser Gln Thr Ala Phe Pro Arg  
                   130                  135

<210> 16  
 <211> 139  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 16  
 Arg Gly Leu Ser Asp Asp Ala Ser His Ala Glu Thr Pro Ser Pro Leu  
   1                  5                  10                  15

Thr Pro Ser Arg Val Asp Ser Phe Ser Asp Gly Val Glu Arg Thr Arg  
                   20                  25                  30

Arg Ser Ser Pro Arg Val Glu Glu His Gln Thr Ser Ser Arg Glu Glu  
                   35                  40                  45

Lys Ala Ala Thr Glu Arg Val Pro Lys Leu Ser Arg Leu Pro Ser Leu  
 50 55 60

Arg Ala Pro Leu Arg Ser Thr Asp Arg Arg Ala Ser Pro Pro Arg Arg  
 65 70 75 80

Leu Ser Gln Leu Leu Arg Cys Cys Thr Thr Ser Arg Phe Ala Ser Lys  
 85 90 95

Gly Thr Ala Tyr Pro Asp Glu Glu Trp Gly His Arg Val Arg Ala Gln  
 100 105 110

Arg Thr Glu Glu Thr Val Ser Ser Leu Thr Thr Lys Arg Leu Leu Thr  
 115 120 125

Arg Ser Pro Asn Ser Gln Thr Ala Phe Pro Arg  
 130 135

<210> 17

<211> 507

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(153)

<400> 17

ggc agg gga agt gga cga cac ccg tcg ctg agc ttt cgc ctg gag tgg 48  
 Gly Arg Gly Ser Gly Arg His Pro Ser Leu Ser Phe Arg Leu Glu Trp  
 1 5 10 15

aga cat cta cct gtg agt gaa cca ggc gtt ctg ctt tcg ccg ctc ctt 96  
 Arg His Leu Pro Val Ser Glu Pro Gly Val Leu Leu Ser Pro Leu Leu  
 20 25 30

tgc agg cca gag gac aat gat aca aat ata agt gac act ctt ctc ttc 144  
 Cys Arg Pro Glu Asp Asn Asp Thr Asn Ile Ser Asp Thr Leu Leu Phe  
 35 40 45

gat atc ggt taactgacaa agaaccacag cggagttaaa atagcagcgt 193  
 Asp Ile Gly  
 50

ttgcagttca acgcatgcac aaactgctta actcccatat gcttgcttt gagagacgcg 253

acagcacatc gttcgagctt gcacgcagcg aagacatcta gacagcaatt aggagatgcc 313  
 tgccgaattt gtatgtaagg cgcaaacgtc tcctcgggtgc gaatcacaat tacgcacatt 373  
 tgcccggact tacatctgtc ttctactggg gtctttcctt gtcaaaccgt gccgctgcaa 433  
 ctccaaacta gctcgtagt gagatgctgg caaggttttg acaagaatcg agttctgcga 493  
 ctgcatcgtg gtcg 507

<210> 18  
 <211> 51  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 18  
 Gly Arg Gly Ser Gly Arg His Pro Ser Leu Ser Phe Arg Leu Glu Trp  
     1                    5                    10                    15  
 Arg His Leu Pro Val Ser Glu Pro Gly Val Leu Leu Ser Pro Leu Leu  
                     20                    25                    30  
 Cys Arg Pro Glu Asp Asn Asp Thr Asn Ile Ser Asp Thr Leu Leu Phe  
                     35                    40                    45  
 Asp Ile Gly  
             50

<210> 19  
 <211> 718  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1) .. (297)

<400> 19  
 gaa ttc cga ctg aat gac tac ctc ttt cag gtg cca gag ggt ccc ccc 48  
 Glu Phe Arg Leu Asn Asp Tyr Leu Phe Gln Val Pro Glu Gly Pro Pro  
     1                    5                    10                    15  
 gcg aga agc cat ggg ttc gac aga aga cga gca gca gcg agc aaa aac 96  
 Ala Arg Ser His Gly Phe Asp Arg Arg Arg Ala Ala Ala Ser Lys Asn  
                     20                    25                    30

gca aca gaa gaa acg cgg agg ctg gcg ggc aaa gaa acg ccg ccg cac 144  
 Ala Thr Glu Glu Thr Arg Arg Leu Ala Gly Lys Glu Thr Pro Pro His  
           35                          40                          45

aga gag gcc ccg gaa aag aca acg cga ggc gaa gaa gac aga caa gag 192  
 Arg Glu Ala Pro Glu Lys Thr Thr Arg Gly Glu Glu Asp Arg Gln Glu  
           50                          55                          60

agc gag agg gaa aga agg cga gcc ggc gtg atg gac aaa aag aac cag 240  
 Ser Glu Arg Glu Arg Arg Arg Ala Gly Val Met Asp Lys Lys Asn Gln  
           65                          70                          75                          80

gac ctt gac gat gaa acc cgg aga agg ggg acg gcg gag gag gag agg 288  
 Asp Leu Asp Asp Glu Thr Arg Arg Arg Gly Thr Ala Glu Glu Glu Arg  
                           85                          90                          95

aat gga gac tgaaaaaagc cgaagatgac aggcacagagt aagacgagga 337  
 Asn Gly Asp

ggtgcaggac aaggatgtct cttattcacc gagtctcggt aaccagcggt ggtcttatca 397

agaggtgcag gacacagatg agacatccgg ttcgtccaaa gaccagttgg agcactcgag 457

agaggcaaga cagaagctga gggttcgcga cagacatcca gctgcctccg cgggcgttgt 517

tcactgagga cttggtcgga aaggggagag aaacatagaa acgaagaaca ccaagacctg 577

gaagaggtgc agattcctct tgggcactcg caggagacgc cttcgtcagt tttttttgtt 637

cactcaacgg actctgtcgt cacgaggga ctcagacaga gacctcaagg agacagagga 697

acgcaacgca cgtcggaatt c 718

<210> 20

<211> 99

<212> PRT

<213> Toxoplasma gondii

<400> 20

Glu Phe Arg Leu Asn Asp Tyr Leu Phe Gln Val Pro Glu Gly Pro Pro  
   1                          5                          10                          15

Ala Arg Ser His Gly Phe Asp Arg Arg Arg Ala Ala Ala Ser Lys Asn  
           20                          25                          30

Ala Thr Glu Glu Thr Arg Arg Leu Ala Gly Lys Glu Thr Pro Pro His  
           35                          40                          45

Arg Glu Ala Pro Glu Lys Thr Thr Arg Gly Glu Glu Asp Arg Gln Glu  
50 55 60

Ser Glu Arg Glu Arg Arg Arg Ala Gly Val Met Asp Lys Lys Asn Gln  
65 70 75 80

Asp Leu Asp Asp Glu Thr Arg Arg Arg Gly Thr Ala Glu Glu Glu Arg  
85 90 95

Asn Gly Asp

<210> 21

<211> 441

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(441)

<400> 21

cgg atc gcc tcg gca ctt cct cat tat ccg tcg cat ggg cat ttc ctg 48  
Arg Ile Ala Ser Ala Leu Pro His Tyr Pro Ser His Gly His Phe Leu  
1 5 10 15

gaa gag gaa caa att ttg ctg ttg gat tgg cag tat caa ctt ggg caa 96  
Glu Glu Glu Gln Ile Leu Leu Leu Asp Trp Gln Tyr Gln Leu Gly Gln  
20 25 30

cga ggc atg gag tcc ggt gta ccc ccc tgc gtg cag cat ggg gat gcg 144  
Arg Gly Met Glu Ser Gly Val Pro Pro Cys Val Gln His Gly Asp Ala  
35 40 45

acg aga agt ttg act tca ccg aaa agg gat gtc agt cat gac ggt cac 192  
Thr Arg Ser Leu Thr Ser Pro Lys Arg Asp Val Ser His Asp Gly His  
50 55 60

caa gga aac agc gga aca aac gca gat gaa gcc ggc caa ggg gcc atg 240  
Gln Gly Asn Ser Gly Thr Asn Ala Asp Glu Ala Gly Gln Gly Ala Met  
65 70 75 80

gca ggc cga gga aag tgc gag tgg agc cgc acc acc ggt gcc aac gta 288  
Ala Gly Arg Gly Lys Cys Glu Trp Ser Arg Thr Thr Gly Ala Asn Val  
85 90 95



ggg tcg tcg tca tgt gtg gtt gat gcg tgt ttg gcg tct gcg ggt aga 336  
 Gly Ser Ser Ser Cys Val Val Asp Ala Cys Leu Ala Ser Ala Gly Arg  
 100 105 110

cat cag gcg gcg agc atg cgt ccg ttt gca cga gat gga ttc ggc gag 384  
 His Gln Ala Ala Ser Met Arg Pro Phe Ala Arg Asp Gly Phe Gly Glu  
 115 120 125

tct act gcg gag aac aga ccc cgt cgg gac ggc gga ctg cca cgt tct 432  
 Ser Thr Ala Glu Asn Arg Pro Arg Arg Asp Gly Gly Leu Pro Arg Ser  
 130 135 140

ctt gga tcg 441  
 Leu Gly Ser  
 145

<210> 22

<211> 147

<212> PRT

<213> Toxoplasma gondii

<400> 22

Arg Ile Ala Ser Ala Leu Pro His Tyr Pro Ser His Gly His Phe Leu  
 1 5 10 15

Glu Glu Glu Gln Ile Leu Leu Leu Asp Trp Gln Tyr Gln Leu Gly Gln  
 20 25 30

Arg Gly Met Glu Ser Gly Val Pro Pro Cys Val Gln His Gly Asp Ala  
 35 40 45

Thr Arg Ser Leu Thr Ser Pro Lys Arg Asp Val Ser His Asp Gly His  
 50 55 60

Gln Gly Asn Ser Gly Thr Asn Ala Asp Glu Ala Gly Gln Gly Ala Met  
 65 70 75 80

Ala Gly Arg Gly Lys Cys Glu Trp Ser Arg Thr Thr Gly Ala Asn Val  
 85 90 95

Gly Ser Ser Ser Cys Val Val Asp Ala Cys Leu Ala Ser Ala Gly Arg  
 100 105 110

His Gln Ala Ala Ser Met Arg Pro Phe Ala Arg Asp Gly Phe Gly Glu  
 115 120 125

Ser Thr Ala Glu Asn Arg Pro Arg Arg Asp Gly Gly Leu Pro Arg Ser

130

135

140

Leu Gly Ser  
145

&lt;210&gt; 23

&lt;211&gt; 428

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(426)

&lt;400&gt; 23

cgg cgg cgt cag cgt gca gac cct tca gac tgg gaa gga tgt gag aat 48  
Arg Arg Arg Gln Arg Ala Asp Pro Ser Asp Trp Glu Gly Cys Glu Asn  
1 5 10 15

gtg gaa aag gat cat ttc ggg agt cgc gag agg cac tcg aat ggg gaa 96  
Val Glu Lys Asp His Phe Gly Ser Arg Glu Arg His Ser Asn Gly Glu  
20 25 30

gag ttc aag aca cag gga aac gtt ggt cga ggt tca ctg agg cag gaa 144  
Glu Phe Lys Thr Gln Gly Asn Val Gly Arg Gly Ser Leu Arg Gln Glu  
35 40 45

ccc ttt acc gat gga gtg tac cac gac agg cag cag cgc ttc tcg gag 192  
Pro Phe Thr Asp Gly Val Tyr His Asp Arg Gln Gln Arg Phe Ser Glu  
50 55 60

aaa gaa cct gcg aag ccg atg ttc act tcc ctc gcg gat ccg agc gtc 240  
Lys Glu Pro Ala Lys Pro Met Phe Thr Ser Leu Ala Asp Pro Ser Val  
65 70 75 80

agg aga cat ttt aag gag gaa gaa gaa cga cgg aaa ttc cag gaa aat 288  
Arg Arg His Phe Lys Glu Glu Glu Glu Arg Arg Lys Phe Gln Glu Lys  
85 90 95

gca gaa gag gag atc ttg cgc ctt ctc aaa cgc gca gct gag tgc agc 336  
Ala Glu Glu Glu Ile Leu Arg Leu Leu Lys Arg Ala Ala Glu Cys Ser  
100 105 110

gag gaa gat ttg aaa agg gaa gaa cgc tcc gaa aag gct acc gaa aag 384  
Glu Glu Asp Leu Lys Arg Glu Glu Arg Ser Glu Lys Ala Thr Glu Lys  
115 120 125

ggg tcc cgt ctc ttc tct gga gag gag gtg cga ttc ttt ccg cc 428  
 Gly Ser Arg Leu Phe Ser Gly Glu Glu Val Arg Phe Phe Pro  
 130 135 140

<210> 24  
 <211> 142  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 24  
 Arg Arg Arg Gln Arg Ala Asp Pro Ser Asp Trp Glu Gly Cys Glu Asn  
 1 5 10 15

Val Glu Lys Asp His Phe Gly Ser Arg Glu Arg His Ser Asn Gly Glu  
 20 25 30

Glu Phe Lys Thr Gln Gly Asn Val Gly Arg Gly Ser Leu Arg Gln Glu  
 35 40 45

Pro Phe Thr Asp Gly Val Tyr His Asp Arg Gln Gln Arg Phe Ser Glu  
 50 55 60

Lys Glu Pro Ala Lys Pro Met Phe Thr Ser Leu Ala Asp Pro Ser Val  
 65 70 75 80

Arg Arg His Phe Lys Glu Glu Glu Glu Arg Arg Lys Phe Gln Glu Lys  
 85 90 95

Ala Glu Glu Glu Ile Leu Arg Leu Leu Lys Arg Ala Ala Glu Cys Ser  
 100 105 110

Glu Glu Asp Leu Lys Arg Glu Glu Arg Ser Glu Lys Ala Thr Glu Lys  
 115 120 125

Gly Ser Arg Leu Phe Ser Gly Glu Glu Val Arg Phe Phe Pro  
 130 135 140

<210> 25  
 <211> 282  
 <212> DNA  
 <213> Toxoplasma gondii

<400> 25  
 cgcgacccgc tgccagtgtt ttgagtctaa ccgccgtatg tcgcggattc cacgtggaaa 60  
 acgacggacc gtcaagacgc ccgagagtgc cgcaatttca cggaccgttc gttcgattcc 120

accaacacct tacgctagcg cttgctagga aacacacatg cgacggcggc tggggcctgg 180

tcgcggatct atccgtacaa tgggagaatc gtctgatgtc tccactgtcc cgctaccgca 240

caggtcctct acaggccaca ccggtagaca gtgagcggcg gc 282

<210> 26

<211> 304

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(303)

<400> 26

cgg act ggc aca gga ccg aag cgc agt tcc tcg aaa ccg acg tcg act 48  
 Arg Thr Gly Thr Gly Pro Lys Arg Ser Ser Ser Lys Pro Thr Ser Thr  
 1 5 10 15

tgg gtc cga ttg tta gtc cat act gaa aca aca atg gaa aac gaa ttg 96  
 Trp Val Arg Leu Leu Val His Thr Glu Thr Thr Met Glu Asn Glu Leu  
 20 25 30

atg aac caa gta agc gac ctc tcg aat gag gct tgg caa aag aaa gaa 144  
 Met Asn Gln Val Ser Asp Leu Ser Asn Glu Ala Trp Gln Lys Lys Glu  
 35 40 45

ctt ccc gtc cta cac aag tgg aca aac agc cct gaa cac tcc ctc ttg 192  
 Leu Pro Val Leu His Lys Trp Thr Asn Ser Pro Glu His Ser Leu Leu  
 50 55 60

aca tcg gaa gac aga gaa aat agt ctt tca aag cca acc gcg gac tca 240  
 Thr Ser Glu Asp Arg Glu Asn Ser Leu Ser Lys Pro Thr Ala Asp Ser  
 65 70 75 80

cca gac agc ttc cgg tat ggc aca cgc aga caa agt cac gca aaa gat 288  
 Pro Asp Ser Phe Arg Tyr Gly Thr Arg Arg Gln Ser His Ala Lys Asp  
 85 90 95

ctg ttc tcc gat ccc g 304  
 Leu Phe Ser Asp Pro  
 100

<210> 27

&lt;211&gt; 101

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 27

Arg Thr Gly Thr Gly Pro Lys Arg Ser Ser Ser Lys Pro Thr Ser Thr

1

5

10

15

Trp Val Arg Leu Leu Val His Thr Glu Thr Thr Met Glu Asn Glu Leu

20

25

30

Met Asn Gln Val Ser Asp Leu Ser Asn Glu Ala Trp Gln Lys Lys Glu

35

40

45

Leu Pro Val Leu His Lys Trp Thr Asn Ser Pro Glu His Ser Leu Leu

50

55

60

Thr Ser Glu Asp Arg Glu Asn Ser Leu Ser Lys Pro Thr Ala Asp Ser

65

70

75

80

Pro Asp Ser Phe Arg Tyr Gly Thr Arg Arg Gln Ser His Ala Lys Asp

85

90

95

Leu Phe Ser Asp Pro

100

&lt;210&gt; 28

&lt;211&gt; 284

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(282)

&lt;400&gt; 28

ccg gac ttc ctc atg tct gaa gat gct tgt ctt gtt cgg ttc gtg cga 48

Pro Asp Phe Leu Met Ser Glu Asp Ala Cys Leu Val Arg Phe Val Arg

1

5

10

15

cac gcg tcg gcc aca cac gcg tat aca cgc agg gca agt gcg agg acg 96

His Ala Ser Ala Thr His Ala Tyr Thr Arg Arg Ala Ser Ala Arg Thr

20

25

30

gta aag ccg ctc aaa ggc caa gga gac aaa gaa cag ggt gcg aca gga 144

Val Lys Pro Leu Lys Gly Gln Gly Asp Lys Glu Gln Gly Ala Thr Gly

35

40

45

aga aat gtt gag gca ata aag aag gaa acc cct ctg aga cgg gaa gcg 192  
 Arg Asn Val Glu Ala Ile Lys Lys Glu Thr Pro Leu Arg Arg Glu Ala  
 50 55 60

aga gaa aac gcg ttt ttt tcg acg ttt tcc ccc gac aga gcg agc gcc 240  
 Arg Glu Asn Ala Phe Phe Ser Thr Phe Ser Pro Asp Arg Ala Ser Ala  
 65 70 75 80

tcc tgt ctc cgc att cac gcg tgt gcc gcg gca gag gaa ccc gg 284  
 Ser Cys Leu Arg Ile His Ala Cys Ala Ala Ala Glu Glu Pro  
 85 90

<210> 29

<211> 94

<212> PRT

<213> Toxoplasma gondii

<400> 29

Pro Asp Phe Leu Met Ser Glu Asp Ala Cys Leu Val Arg Phe Val Arg  
 1 5 10 15

His Ala Ser Ala Thr His Ala Tyr Thr Arg Arg Ala Ser Ala Arg Thr  
 20 25 30

Val Lys Pro Leu Lys Gly Gln Gly Asp Lys Glu Gln Gly Ala Thr Gly  
 35 40 45

Arg Asn Val Glu Ala Ile Lys Lys Glu Thr Pro Leu Arg Arg Glu Ala  
 50 55 60

Arg Glu Asn Ala Phe Phe Ser Thr Phe Ser Pro Asp Arg Ala Ser Ala  
 65 70 75 80

Ser Cys Leu Arg Ile His Ala Cys Ala Ala Ala Glu Glu Pro  
 85 90

<210> 30

<211> 690

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(690)

&lt;400&gt; 30

cga cgt ccc tac cac tat gaa atg ttg gac atc ccg agc atc cgg cgt	48
Arg Arg Pro Tyr His Tyr Glu Met Leu Asp Ile Pro Ser Ile Arg Arg	
1 5 10 15	
gtg gag ttg cca ggt gcg cag gtc cgt atg cca atg gcc aaa gag ctc	96
Val Glu Leu Pro Gly Ala Gln Val Arg Met Pro Met Ala Lys Glu Leu	
20 25 30	
gta cgc gat tgg ggt tct gtc gtc cag cag cag acg act tct gat tct	144
Val Arg Asp Trp Gly Ser Val Val Gln Gln Gln Thr Thr Ser Asp Ser	
35 40 45	
tct agt gac aca cca gct acc cgc agt cgc tct gct gaa gca ctc tgt	192
Ser Ser Asp Thr Pro Ala Thr Arg Ser Arg Ser Ala Glu Ala Leu Cys	
50 55 60	
gtc ttt tcg acg cct tgt aca gca gac agc gac caa cgt atg aaa ggc	240
Val Phe Ser Thr Pro Cys Thr Ala Asp Ser Asp Gln Arg Met Lys Gly	
65 70 75 80	
cgc cat tac cca cag tca tat cat acg ccg agg gac agc gcc acc aaa	288
Arg His Tyr Pro Gln Ser Tyr His Thr Pro Arg Asp Ser Ala Thr Lys	
85 90 95	
aga gaa aaa cct ctc aaa agt aca ttt atc tgg ggc act aca gtg gaa	336
Arg Glu Lys Pro Leu Lys Ser Thr Phe Ile Trp Gly Thr Thr Val Glu	
100 105 110	
gac aga aac cac ccc atc agc cca gac ccg ttc tca agg ctg cag gga	384
Asp Arg Asn His Pro Ile Ser Pro Asp Pro Phe Ser Arg Leu Gln Gly	
115 120 125	
tgt ggc cag acc ctc cag gac gag ctc cca tca gct cgc act aga ccg	432
Cys Gly Gln Thr Leu Gln Asp Glu Leu Pro Ser Ala Arg Thr Arg Pro	
130 135 140	
gga tgg gcc gca ttg gac tcc cgc ctg aaa aac aag gac ccg cag att	480
Gly Trp Ala Ala Leu Asp Ser Arg Leu Lys Asn Lys Asp Pro Gln Ile	
145 150 155 160	
agc gca gga gac gaa gcc gcg aag gtc gac gac acg tca gcg gaa cct	528
Ser Ala Gly Asp Glu Ala Ala Lys Val Asp Asp Thr Ser Ala Glu Pro	
165 170 175	
tgc ctg gga acg gta ccg tcc ttt tgt cgg ctt gta aca agt cac gac	576
Cys Leu Gly Thr Val Pro Ser Phe Cys Arg Leu Val Thr Ser His Asp	
180 185 190	

ttg cta gag gct gga gcg cag gtt cgt gtg ctt ggg cca acg aca gac 624  
 Leu Leu Glu Ala Gly Ala Gln Val Arg Val Leu Gly Pro Thr Thr Asp  
           195                          200                          205

ccg gag aca gag acc gct tct cag ctc cag aca act gag ctt gcc acg 672  
 Pro Glu Thr Glu Thr Ala Ser Gln Leu Gln Thr Thr Glu Leu Ala Thr  
           210                          215                          220

ctg aca act gtg gat ccg 690  
 Leu Thr Thr Val Asp Pro  
           225                          230

<210> 31  
 <211> 230  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 31  
 Arg Arg Pro Tyr His Tyr Glu Met Leu Asp Ile Pro Ser Ile Arg Arg  
       1                          5                          10                          15

Val Glu Leu Pro Gly Ala Gln Val Arg Met Pro Met Ala Lys Glu Leu  
                           20                          25                          30

Val Arg Asp Trp Gly Ser Val Val Gln Gln Gln Thr Thr Ser Asp Ser  
           35                          40                          45

Ser Ser Asp Thr Pro Ala Thr Arg Ser Arg Ser Ala Glu Ala Leu Cys  
           50                          55                          60

Val Phe Ser Thr Pro Cys Thr Ala Asp Ser Asp Gln Arg Met Lys Gly  
       65                          70                          75                          80

Arg His Tyr Pro Gln Ser Tyr His Thr Pro Arg Asp Ser Ala Thr Lys  
                           85                          90                          95

Arg Glu Lys Pro Leu Lys Ser Thr Phe Ile Trp Gly Thr Thr Val Glu  
           100                          105                          110

Asp Arg Asn His Pro Ile Ser Pro Asp Pro Phe Ser Arg Leu Gln Gly  
           115                          120                          125

Cys Gly Gln Thr Leu Gln Asp Glu Leu Pro Ser Ala Arg Thr Arg Pro  
           130                          135                          140

Gly Trp Ala Ala Leu Asp Ser Arg Leu Lys Asn Lys Asp Pro Gln Ile



145                      150                      155                      160  
 Ser Ala Gly Asp Glu Ala Ala Lys Val Asp Asp Thr Ser Ala Glu Pro  
                                  165                      170                      175  
 Cys Leu Gly Thr Val Pro Ser Phe Cys Arg Leu Val Thr Ser His Asp  
                                  180                      185                      190  
 Leu Leu Glu Ala Gly Ala Gln Val Arg Val Leu Gly Pro Thr Thr Asp  
                                  195                      200                      205  
 Pro Glu Thr Glu Thr Ala Ser Gln Leu Gln Thr Thr Glu Leu Ala Thr  
                                  210                      215                      220  
 Leu Thr Thr Val Asp Pro  
 225                      230

<210> 32  
 <211> 313  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(162)

<400> 32  
 cgc agg aat aat cct gac ggt cag acg cag cgg ttc gtg cag aca gtg 48  
 Arg Arg Asn Asn Pro Asp Gly Gln Thr Gln Arg Phe Val Gln Thr Val  
   1                                 5                                 10                                 15  
 aag caa tgg cag agt gta aaa agc aga acc aga gcg tgt ctg tcg gcc 96  
 Lys Gln Trp Gln Ser Val Lys Ser Arg Thr Arg Ala Cys Leu Ser Ala  
                                  20                                 25                                 30  
 aaa gga aag aga agg caa atc aca cag cga ata aac ctc acc tct gtc 144  
 Lys Gly Lys Arg Arg Gln Ile Thr Gln Arg Ile Asn Leu Thr Ser Val  
                                  35                                 40                                 45  
 tcg cac ccc gaa gca acg taggagagcc actggtgccg ccactctgtg 192  
 Ser His Pro Glu Ala Thr  
                                  50  
 ctgacaaaaa agaaccggcc cttcttcggc aggggcgtag ccagtctgca gacatttcaa 252  
 tttcgaagcg accggaagca gtgaaatttc caggggaagac gcccaggaga cgtcaacagc 312

g

313

<210> 33  
 <211> 54  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 33  
 Arg Arg Asn Asn Pro Asp Gly Gln Thr Gln Arg Phe Val Gln Thr Val  
 1 5 10 15

Lys Gln Trp Gln Ser Val Lys Ser Arg Thr Arg Ala Cys Leu Ser Ala  
 20 25 30

Lys Gly Lys Arg Arg Gln Ile Thr Gln Arg Ile Asn Leu Thr Ser Val  
 35 40 45

Ser His Pro Glu Ala Thr  
 50

<210> 34  
 <211> 389  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(195)

<400> 34  
 cgc tct cac gga ggc gca agt gag ttt tgg ctt tac ctc ttg aga aaa 48  
 Arg Ser His Gly Gly Ala Ser Glu Phe Trp Leu Tyr Leu Leu Arg Lys  
 1 5 10 15

cgg aac tct cca gaa gat cct cgt tcc gtc cgt cct cca cgt ccg tgt 96  
 Arg Asn Ser Pro Glu Asp Pro Arg Ser Val Arg Pro Pro Arg Pro Cys  
 20 25 30

gtc ttt cga gag atg gac aaa cag aga agc aga atc aag aaa gga ttc 144  
 Val Phe Arg Glu Met Asp Lys Gln Arg Ser Arg Ile Lys Lys Gly Phe  
 35 40 45

gca ttt gca ctt ggg tct gtc ttt tac ttc caa ggt cgt gaa ttt cat 192  
 Ala Phe Ala Leu Gly Ser Val Phe Tyr Phe Gln Gly Arg Glu Phe His  
 50 55 60

gcg tgacgaataa gagagacagg agtagggcgc aactttctgt ctcttggcag 245  
 Ala  
 65

tttccgattt ctcttccttc cgaagccctt gctgccaaagc actccatccg gtccgggttg 305

tctctctcag gttcttcgag caatcgacgc gatgttctct gctgtcgatg cgggggcttg 365

gcgtgtctgc atatctcttc cagg 389

<210> 35

<211> 65

<212> PRT

<213> Toxoplasma gondii

<400> 35

Arg Ser His Gly Gly Ala Ser Glu Phe Trp Leu Tyr Leu Leu Arg Lys  
 1 5 10 15

Arg Asn Ser Pro Glu Asp Pro Arg Ser Val Arg Pro Pro Arg Pro Cys  
 20 25 30

Val Phe Arg Glu Met Asp Lys Gln Arg Ser Arg Ile Lys Lys Gly Phe  
 35 40 45

Ala Phe Ala Leu Gly Ser Val Phe Tyr Phe Gln Gly Arg Glu Phe His  
 50 55 60

Ala

65

<210> 36

<211> 548

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(546)

<400> 36

cga tct tct tct cac cgt tgc ctc ttc ttt ctc tcc gtt gtc tgc gtc 48  
 Arg Ser Ser Ser His Arg Ser Leu Phe Phe Leu Ser Val Val Cys Val  
 1 5 10 15

ctc tcc cca ctg cct ctc gcc gtc cgc gtc gtt cgc ctc cgg gga agc 96

Leu Ser Pro Leu Pro Leu Ala Val Arg Val Val Arg Leu Arg Gly Ser  
                   20                                  25                                  30

cgg cag tgt ggc gag cac ggc ggc ttc gct cga aga gca gcg cct cgc 144  
 Arg Gln Cys Gly Glu His Gly Gly Phe Ala Arg Arg Ala Ala Pro Arg  
                   35                                  40                                  45

gcg ttc ctt cgg gga cgc ccg aca agc ctg cgt tca tcc cag aga acg 192  
 Ala Phe Leu Arg Gly Arg Pro Thr Ser Leu Arg Ser Ser Gln Arg Thr  
                   50                                  55                                  60

cct cgg tct gcg caa atg cgc cgt cgc tcc cca cac atg aga tgc ttt 240  
 Pro Arg Ser Ala Gln Met Arg Arg Arg Ser Pro His Met Arg Cys Phe  
                   65                                  70                                  75                                  80

tgc gag act ggc agc agc gcc tgt tgc gag cga agg aag agg agc gcg 288  
 Cys Glu Thr Gly Ser Ser Ala Cys Cys Glu Arg Arg Lys Arg Ser Ala  
                                   85                                  90                                  95

agg gat ggc aac ctc cag gag agc gcg aag aag gcc cgt ccc tcg aat 336  
 Arg Asp Gly Asn Leu Gln Glu Ser Ala Lys Lys Ala Arg Pro Ser Asn  
                   100                                  105                                  110

ccg atg agc aag gca atc cat gct tca gtc gac cga gtc cag tgc ggt 384  
 Pro Met Ser Lys Ala Ile His Ala Ser Val Asp Arg Val Gln Cys Gly  
                   115                                  120                                  125

caa cag gac tcg aaa agg tcg agg aga tgg ccg gcg gct tcg act tct 432  
 Gln Gln Asp Ser Lys Arg Ser Arg Arg Trp Pro Ala Ala Ser Thr Ser  
                   130                                  135                                  140

gcg ggg gtg cag gcg att cgt gga aga aac agc gaa gtc ccg agg gtg 480  
 Ala Gly Val Gln Ala Ile Arg Gly Arg Asn Ser Glu Val Pro Arg Val  
                   145                                  150                                  155                                  160

gac agg tcc gcc aag tcg cct acg cca ctc tcg aag aag ccg aaa atg 528  
 Asp Arg Ser Ala Lys Ser Pro Thr Pro Leu Ser Lys Lys Pro Lys Met  
                   165                                  170                                  175

cgg tct ctg ccg cat acg gc 548  
 Arg Ser Leu Pro His Thr  
                   180

&lt;210&gt; 37

&lt;211&gt; 182

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 37

Arg Ser Ser Ser His Arg Ser Leu Phe Phe Leu Ser Val Val Cys Val  
 1 5 10 15

Leu Ser Pro Leu Pro Leu Ala Val Arg Val Val Arg Leu Arg Gly Ser  
 20 25 30

Arg Gln Cys Gly Glu His Gly Gly Phe Ala Arg Arg Ala Ala Pro Arg  
 35 40 45

Ala Phe Leu Arg Gly Arg Pro Thr Ser Leu Arg Ser Ser Gln Arg Thr  
 50 55 60

Pro Arg Ser Ala Gln Met Arg Arg Arg Ser Pro His Met Arg Cys Phe  
 65 70 75 80

Cys Glu Thr Gly Ser Ser Ala Cys Cys Glu Arg Arg Lys Arg Ser Ala  
 85 90 95

Arg Asp Gly Asn Leu Gln Glu Ser Ala Lys Lys Ala Arg Pro Ser Asn  
 100 105 110

Pro Met Ser Lys Ala Ile His Ala Ser Val Asp Arg Val Gln Cys Gly  
 115 120 125

Gln Gln Asp Ser Lys Arg Ser Arg Arg Trp Pro Ala Ala Ser Thr Ser  
 130 135 140

Ala Gly Val Gln Ala Ile Arg Gly Arg Asn Ser Glu Val Pro Arg Val  
 145 150 155 160

Asp Arg Ser Ala Lys Ser Pro Thr Pro Leu Ser Lys Lys Pro Lys Met  
 165 170 175

Arg Ser Leu Pro His Thr  
 180

&lt;210&gt; 38

&lt;211&gt; 310

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

<223> At locations 16, 24, 25, 31, 38, 45 and 57, Xaa =  
 unknown

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(285)

&lt;400&gt; 38

cgg gat cca gct gca cct aac agc aca cag gct gtg gca gcc gct ygt 48  
 Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Ala Xaa  
 1 5 10 15

acc gtg gta gtg atg aaa acm gam gmw gaa gtg tcc ggt gac aac stc 96  
 Thr Val Val Val Met Lys Xaa Xaa Xaa Glu Val Ser Gly Asp Asn Xaa  
 20 25 30

agt caa ccg ggt agg sgt ccg ccg tcg cca aag ccg caw acg acg aag 144  
 Ser Gln Pro Gly Arg Xaa Pro Pro Ser Pro Lys Pro Xaa Thr Thr Lys  
 35 40 45

ttt ccg ccg aga gag tca cca gac srg cag ggg acg agg ccg aga act 192  
 Phe Pro Arg Arg Glu Ser Pro Asp Xaa Gln Gly Thr Arg Arg Arg Thr  
 50 55 60

gaa agc cga ggc gct gtt agc agg gta tgg cca ggg gaa aac caq mqa 240  
 Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Xaa  
 65 70 75 80

aga ctg tct gcc gtc gac gat tcg ata ccg gct aac cca tcg ctt 285  
 Arg Leu Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ser Leu  
 85 90 95

tgaacgggtg gcgccctgag atccg 310

&lt;210&gt; 39

&lt;211&gt; 95

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 39

Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Ala Xaa  
 1 5 10 15

Thr Val Val Val Met Lys Xaa Xaa Xaa Glu Val Ser Gly Asp Asn Xaa  
 20 25 30

Ser Gln Pro Gly Arg Xaa Pro Pro Ser Pro Lys Pro Xaa Thr Thr Lys  
 35 40 45

Phe Pro Arg Arg Glu Ser Pro Asp Xaa Gln Gly Thr Arg Arg Arg Thr

50

55

60

Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Xaa  
 65 70 75 80

Arg Leu Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ser Leu  
 85 90 95

&lt;210&gt; 40

&lt;211&gt; 220

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(219)

&lt;400&gt; 40

cgg gat cct tgc ctc agt gtc agg gac atc gag cgt atg ttc cgt ata 48  
 Arg Asp Pro Cys Leu Ser Val Arg Asp Ile Glu Arg Met Phe Arg Ile  
 1 5 10 15

tgt cac cat cgt tct ctg tct cgc ctc ctt ggc gcc tct gtt gct tgg 96  
 Cys His His Arg Ser Leu Ser Arg Leu Leu Gly Ala Ser Val Ala Trp  
 20 25 30

gat gca gtt gac tgc tct tcg gct tcg tcg cgc aca cac tgg tcc ttg 144  
 Asp Ala Val Asp Cys Ser Ser Ala Ser Ser Arg Thr His Trp Ser Leu  
 35 40 45

ctt gcg tct gag ctc cct tcc gaa cgg gtt ctt ttt cga ctg cag gtt 192  
 Leu Ala Ser Glu Leu Pro Ser Glu Arg Val Leu Phe Arg Leu Gln Val  
 50 55 60

ctt cta aaa ttg cca gtt ccc gat ccc g 220  
 Leu Leu Lys Leu Pro Val Pro Asp Pro  
 65 70

&lt;210&gt; 41

&lt;211&gt; 73

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 41

Arg Asp Pro Cys Leu Ser Val Arg Asp Ile Glu Arg Met Phe Arg Ile  
 1 5 10 15

Cys His His Arg Ser Leu Ser Arg Leu Leu Gly Ala Ser Val Ala Trp  
                   20                                  25                                  30

Asp Ala Val Asp Cys Ser Ser Ala Ser Ser Arg Thr His Trp Ser Leu  
                   35                                  40                                  45

Leu Ala Ser Glu Leu Pro Ser Glu Arg Val Leu Phe Arg Leu Gln Val  
                   50                                  55                                  60

Leu Leu Lys Leu Pro Val Pro Asp Pro  
                   65                                  70

<210> 42

<211> 642

<212> DNA

<213> *Toxoplasma gondii*

<220>

<223> N= unknown at 19, 23, 27, 28, 29, 41, 86 and 88

<220>

<223> Xaa = unknown at 7, 8, 9, 10, 14, 29 and 30

<220>

<221> CDS

<222> (1)..(102)

<400> 42

cgg cgg gaa acc atg gag ntt tna tan nnt aca act tcc ana tgc atg 48  
 Arg Arg Glu Thr Met Glu Xaa Xaa Xaa Xaa Thr Thr Ser Xaa Cys Met  
           1                                  5                                  10                                  15

gta ggt acc caa aat gca aac tat aga cac aaa caa ang naa aat acn 96  
 Val Gly Thr Gln Asn Ala Asn Tyr Arg His Lys Gln Xaa Xaa Asn Xaa  
                   20                                  25                                  30

tgg ggg tgatgcanna nggggangtn ggggacagan aaatngtcct tcagttntca 152  
 Trp Gly

tctttgcccg cngcgtngan nacgcaatac agcggggcgca gcggtcatc acaccantac 212

acgantnttg caaagaagca cntntcttct ctcttcangt ctctntacca cttctaccac 272

ctgcaccccc gcttcgtcca caaaacacat ttgaacgatg tgaccaaagt gatccacaaa 332

aacacgattg tttcgtcaca tgaaacctca gcaaattcag gcgccaggac ggctccttca 392



aacgtctaata ccagagtcct ctccgctcaa aaacacgatt gtttcgtcac atggaacctc 452  
 agcaaattca ggcgccagga cggcctccct tcaaacgten taatccagag tcntttccgn 512  
 tccatcccca cnttntgccc ntacacgttt ccagtgggtgg catgtcatcg tctccccctg 572  
 tcaacgtccc atcacctgag tacaggcgcg aagcagcgga cagctgttct tccatctccc 632  
 tgtattccgg 642

<210> 43  
 <211> 34  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 43  
 Arg Arg Glu Thr Met Glu Xaa Xaa Xaa Xaa Thr Thr Ser Xaa Cys Met  
 1 5 10 15  
 Val Gly Thr Gln Asn Ala Asn Tyr Arg His Lys Gln Xaa Xaa Asn Xaa  
 20 25 30

Trp Gly

<210> 44  
 <211> 381  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(81)

<400> 44  
 cgg atc cac aaa aac acg att gtt tcg tca cat gga acc tca gca aat 48  
 Arg Ile His Lys Asn Thr Ile Val Ser Ser His Gly Thr Ser Ala Asn  
 1 5 10 15  
 tca ggc gcc agg acg gcc tcc ctt caa acg tcc taatccagag tcctctccgc 101  
 Ser Gly Ala Arg Thr Ala Ser Leu Gln Thr Ser  
 20 25  
 tccatcccca ccttctgccc cttcacgttt ccagtgggtgg catgtcatcg tctccccctg 161

tcaacgtccc atcacctgag tacaggcgcg aagcagcgga cagctgttct tccatctccc 221  
 tgtattccgg agtctctatc gcttgcaagg cgagcaggcg ggcctcgaca gaagggttaa 281  
 tcaacttgta aaccagaagt ttcacgttct ctggcaccg cgggcacctc gaaaaaaaga 341  
 attcgacact gtattcgtag cccgattttg tarcggagg 381

<210> 45  
 <211> 27  
 <212> PRT  
 <213> *Toxoplasma gondii*

<400> 45  
 Arg Ile His Lys Asn Thr Ile Val Ser Ser His Gly Thr Ser Ala Asn  
 1 5 10 15  
 Ser Gly Ala Arg Thr Ala Ser Leu Gln Thr Ser  
 20 25

<210> 46  
 <211> 432  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <221> CDS  
 <222> (1)..(255)

<400> 46  
 ttt ttt tcg agg tgc cgg cgg gtg cca gag aac gtg aaa ctt ctg gtt 48  
 Phe Phe Ser Arg Cys Arg Arg Val Pro Glu Asn Val Lys Leu Leu Val  
 1 5 10 15  
 tac aag ttg att aac cct tct gtc gag gcc cgc ctg ctc gcc ttg caa 96  
 Tyr Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Leu Ala Leu Gln  
 20 25 30  
 gcg ata gag act ccg gaa tac agg gag atg gaa gaa cag ctg tcc gct 144  
 Ala Ile Glu Thr Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala  
 35 40 45  
 gct tcg cgc ctg tac tca ggt gat ggg acg ttg aca ggg gga gac gat 192  
 Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp  
 50 55 60

gac atg cca cca ctg aaa cgt gaa ggg gca gaa ggt ggg gat gga gcg 240  
 Asp Met Pro Pro Leu Lys Arg Glu Gly Ala Glu Gly Gly Asp Gly Ala  
 65 70 75 80

gag agg act ctg gat taggacgttt gaagggaggc cgtcctggcg cctgaatttg 295  
 Glu Arg Thr Leu Asp  
 85

ctgaggttcc atgtgacgaa acaatcgtgt ttttgagcgg agaggactct ggattaggac 355  
 gtttgaaggg aggccgtcct ggcgcctgaa tttgctgagg tttcatgtga cgaaacaatc 415  
 gtgtttttgt ggatccg 432

<210> 47

<211> 85

<212> PRT

<213> Toxoplasma gondii

<400> 47

Phe Phe Ser Arg Cys Arg Arg Val Pro Glu Asn Val Lys Leu Leu Val  
 1 5 10 15

Tyr Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Leu Ala Leu Gln  
 20 25 30

Ala Ile Glu Thr Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala  
 35 40 45

Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp  
 50 55 60

Asp Met Pro Pro Leu Lys Arg Glu Gly Ala Glu Gly Gly Asp Gly Ala  
 65 70 75 80

Glu Arg Thr Leu Asp  
 85

<210> 48

<211> 282

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(105)

&lt;400&gt; 48

cgg cgg gct gct tcc cag gaa cgt ttc gcg gct gcg tgt gga cag caa 48  
 Arg Arg Ala Ala Ser Gln Glu Arg Phe Ala Ala Ala Cys Gly Gln Gln  
 1 5 10 15

agc ctt acc ctc gag ttt tct ctc gtg gct gcc gac gtc ggc gac gcc 96  
 Ser Leu Thr Leu Glu Phe Ser Leu Val Ala Ala Asp Val Gly Asp Ala  
 20 25 30

gcg aac tcc tgagatcaaa cacacaaaaa ggccctcggt gaaacatccc 145  
 Ala Asn Ser  
 35

cacgcacgag cagaaggacg cgagcaagaa aacgtctcca gccttctctt gcggtcgctt 205

gcaagcgga gtgtcgtctc cctctgtctt tctctgtgta ctgaagccc agcgacttcc 265

ttgtcgagtt tctccgg 282

&lt;210&gt; 49

&lt;211&gt; 35

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 49

Arg Arg Ala Ala Ser Gln Glu Arg Phe Ala Ala Ala Cys Gly Gln Gln  
 1 5 10 15

Ser Leu Thr Leu Glu Phe Ser Leu Val Ala Ala Asp Val Gly Asp Ala  
 20 25 30

Ala Asn Ser  
 35

&lt;210&gt; 50

&lt;211&gt; 466

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;223&gt; Xaa = unknown at 3, 11 and 36

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(213)

&lt;400&gt; 50

ttt ttt tsg agg tgc cgg cgg gtg cca gag aam gtg aaa ttc tgg ttt 48  
 Phe Phe Xaa Arg Cys Arg Arg Val Pro Glu Xaa Val Lys Phe Trp Phe  
 1 5 10 15

aac aag ttg att aac cct tct gtc gag gcc cgc ctg ttc gcc ttg caa 96  
 Asn Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Phe Ala Leu Gln  
 20 25 30

gcg ata gag ayt ccg gaa tac agg gag atg gaa gaa cag ctg tcc gct 144  
 Ala Ile Glu Xaa Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala  
 35 40 45

gct tcg cgc ctg tac tca ggt gat ggg acg ttg aca ggg gga gac gat 192  
 Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp  
 50 55 60

gac atg cca cca ctg gaa acg tgaaggggca gaaggtgggg atggagcgga 243  
 Asp Met Pro Pro Leu Glu Thr  
 65 70

gaggactctg gattaggacg tttgaaggga ggccgtcctg gcgcctgaat ttgctgaggt 303

tccatgtgac gaaacaatcg tgtttttgag cggagaggac tctggattag gacgtttgaa 363

gggaggccgt cctggcgcct gaatttgctg aggtttcatg tgacgaaaca atcgtgtttt 423

tgtggatcct cccgatacaa aatcggggta cgaatacagt gtc 466

&lt;210&gt; 51

&lt;211&gt; 71

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 51

Phe Phe Xaa Arg Cys Arg Arg Val Pro Glu Xaa Val Lys Phe Trp Phe  
 1 5 10 15

Asn Lys Leu Ile Asn Pro Ser Val Glu Ala Arg Leu Phe Ala Leu Gln  
 20 25 30

Ala Ile Glu Xaa Pro Glu Tyr Arg Glu Met Glu Glu Gln Leu Ser Ala  
 35 40 45

Ala Ser Arg Leu Tyr Ser Gly Asp Gly Thr Leu Thr Gly Gly Asp Asp  
 50 55 60

Asp Met Pro Pro Leu Glu Thr  
65 70

<210> 52

<211> 539

<212> DNA

<213> Toxoplasma gondii

<220>

<223> Xaa = unknown at 8, 9 and 16

<220>

<221> CDS

<222> (1)..(60)

<400> 52

gat agc aca cgg aat gga tgc ntg grg gtt ggg agc gac tat att tnt 48  
Asp Ser Thr Arg Asn Gly Cys Xaa Xaa Val Gly Ser Asp Tyr Ile Xaa  
1 5 10 15

tat ttg gtg ctt taaagctcca actacaggac ctgaagagga atactccatc 100  
Tyr Leu Val Leu  
20

gaattcttgt tctcattgtg ccggcgggca ccagagaacg tgaaactact ggtttacaag 160

ttgattaacc cttctgtcga ggcccgcctg tcgccttgca agctacggag actccggaat 220

acagggagat ggaagaacag ctgtccgctg cttcgcgcct gtactcaggt gatgggacgt 280

tgacaggggg agacgatgac atgccaccac cggaaacgtg aaggggcaga aggtggggat 340

ggagcggaga ggactctgga ttaggacgtt tgaaggagg cgcctctggc gcctgaattt 400

tgctgagggt tcatgtgacg aaacaatcgt gtttttgtgg atccggaatt ccggatcggg 460

gaatttcctc tcacaccgct tggggccgag acacgcgcag agacgttggt gggcctccac 520

aacacagggg ggattaagg 539

<210> 53

<211> 20

<212> PRT

<213> Toxoplasma gondii

&lt;400&gt; 53

Asp Ser Thr Arg Asn Gly Cys Xaa Xaa Val Gly Ser Asp Tyr Ile Xaa  
 1 5 10 15

Tyr Leu Val Leu  
 20

&lt;210&gt; 54

&lt;211&gt; 1233

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 54

cgggatccct gaaggagagc atattcctga agagttccca gaaggcgagc atgttcctga 60  
 ggaggaaatc cctgaaggag aacatattcc tgaggaggag ttccctgaag gagagcatgt 120  
 tcctgaggag gagatccctg aaggcgagca tgttcctgag gaggagctcc ctggaggaga 180  
 acttattcct gaggaggaga tccctgaagg agagcatgtt cctgaagagc tccctgaagg 240  
 cgagcatgtt cctgaggagg agatccctga aggagagcat gttcctgaag aggaaatccc 300  
 tgaaggcgag catgttcctg aggaggagat ccctgaagga gaacatgctc cagaggaaga 360  
 gactcctgca cctgaggaga ccgaaaagga ggaggaagaa ggcgtgccag tcgcagcgat 420  
 tgccggtggt gtcgtcgag gtgtgttgt cattgctggt ggtgcagggt ctgccgtgta 480  
 cacaaccaa ggtggcgttg aagcagctga agacgaagt atgtttgaga gcgaagaaga 540  
 cggaaccag gctggcgaga accgcgagag gagacggtca ttgagatcga agatgacgca 600  
 tgggcagaca ttggactaaa ggagactagg aggtctgtgt gggcacatgc aggcgtgcga 660  
 caaaaccgtg atcgcgaggt attctgtgtt acgggcggag cgtctgcggc tgtccttcga 720  
 aggggaggcg gagtgacact ctgagctagg taccagacga acgcagccat ttgtgtccgt 780  
 ccgctgtttc ttgatcctgg acacagacca gacccgacac ggggtgctgaa cggtaatgca 840  
 aactgtcggg aaaacctctc cggcgcgaaa acagagattt acacaccgtt gagatctgag 900  
 tagcggaaagt gcatgcgat gtgtgtccac gagaaaggaa gattttcttt cgagaacgtt 960  
 tccctttgtt cgcacattcc tacgcggggc tcgtgccgca tgcattgcga gaggactgcg 1020

ttcgagtgtt ctttcgccgt tgcagtgtgt gacattgcgg cgtgggcaaa ggatagaagt 1080  
 gacgacctct gacatggcag tgaaggtggc agagactcgc ggaaaatcca aaaactctct 1140  
 gccgtttcgg tgcaggaatc acctttcttt ttttcgtctc tggaccgcc tccgtggtgt 1200  
 tcccttgccc ttgcaagcgg ctgctatgta gcg 1233

<210> 55  
 <211> 411  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(180)

<400> 55  
 cga cga cct cgg ctt ctc cac ata caa gga atg tct tcc tgt ttt gga 48  
 Arg Arg Pro Arg Leu Leu His Ile Gln Gly Met Ser Ser Cys Phe Gly  
 1 5 10 15  
 cct aag caa ccc gac ctt tat ctt ttg cac cag ctg tgc ttc ttt tac 96  
 Pro Lys Gln Pro Asp Leu Tyr Leu Leu His Gln Leu Cys Phe Tyr  
 20 25 30  
 ttg tgt gaa tca ctg tgt aaa caa act gag aag cgt gta tgc atg gtc 144  
 Leu Cys Glu Ser Leu Cys Lys Gln Thr Glu Lys Arg Val Cys Met Val  
 35 40 45  
 gcc ttt gca tgt gga cga ggc cgc cgt cgc aca gcg tgattctcat 190  
 Ala Phe Ala Cys Gly Arg Gly Arg Arg Arg Thr Ala  
 50 55 60  
 ctctgttgcg tgggggcgcg gatgagaatc aactccttag tgtcacagca tcagtgcagt 250  
 gcgtggagca acaattcttt tcgtgcacag acaagacaca ccagatatga aagaacacta 310  
 acgggcactt accgttgtcc gtctatatat ttatatttag tcaatgctga gattagacct 370  
 agacttgatga gagagagtgt gaaacccaaa tgccatagatc c 411

<210> 56  
 <211> 60  
 <212> PRT  
 <213> Toxoplasma gondii



&lt;400&gt; 56

Arg Arg Pro Arg Leu Leu His Ile Gln Gly Met Ser Ser Cys Phe Gly  
 1 5 10 15

Pro Lys Gln Pro Asp Leu Tyr Leu Leu His Gln Leu Cys Phe Phe Tyr  
 20 25 30

Leu Cys Glu Ser Leu Cys Lys Gln Thr Glu Lys Arg Val Cys Met Val  
 35 40 45

Ala Phe Ala Cys Gly Arg Gly Arg Arg Arg Thr Ala  
 50 55 60

&lt;210&gt; 57

&lt;211&gt; 441

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;223&gt; Xaa = unknown at 51, 80 and 109

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(354)

&lt;400&gt; 57

cgg atc gaa gaa gct gaa gcg gag aca cga atc gcc gag aca ggc aaa 48  
 Arg Ile Glu Glu Ala Glu Ala Glu Thr Arg Ile Ala Glu Thr Gly Lys  
 1 5 10 15

cac agc ggg aat gag aat cga ctc tgc gat aga agt ggg cgc cat gga 96  
 His Ser Gly Asn Glu Asn Arg Leu Cys Asp Arg Ser Gly Arg His Gly  
 20 25 30

atc aag gaa ccg agg cga agg agg ccc atg ctg ttg gcc gag gtg ccc 144  
 Ile Lys Glu Pro Arg Arg Arg Arg Pro Met Leu Leu Ala Glu Val Pro  
 35 40 45

tgc ttg tkg gag ggc gcc cga cga aca ggg ttt cgt cag aga caa gaa 192  
 Cys Leu Xaa Glu Gly Ala Arg Arg Thr Gly Phe Arg Gln Arg Gln Ala  
 50 55 60

ctt cgc tcg cgt ttg tgg ccc ctt gcc gtg cgg cac gcg tgc gta kcc 240  
 Leu Arg Ser Arg Leu Trp Pro Leu Ala Val Arg His Ala Cys Val Xaa  
 65 70 75 80

ttc aag aga gac tgc gga agc aga gag agg cca ttg agg ctg tcc gag 288  
 Phe Lys Arg Asp Cys Gly Ser Arg Glu Arg Pro Leu Arg Leu Ser Glu  
                     85                                    90                                    95

gtc ggc tcc agc cga gct gga tcc gaa tcc tgc agc csg gga tcc act 336  
 Val Gly Ser Ser Arg Ala Gly Ser Glu Ser Cys Ser Xaa Gly Ser Thr  
                     100                                    105                                    110

agt cta gac gcg cac ccg tgacccactt caggaygcgg vmatwatrcm 384  
 Ser Leu Asp Ala His Pro  
                     115

ggggcagatt tttwmgyta actatcattt cccstwggtt gattmtcca gcaattg 441

<210> 58

<211> 118

<212> PRT

<213> Toxoplasma gondii

<400> 58

Arg Ile Glu Glu Ala Glu Ala Glu Thr Arg Ile Ala Glu Thr Gly Lys  
     1                    5                                    10                                    15

His Ser Gly Asn Glu Asn Arg Leu Cys Asp Arg Ser Gly Arg His Gly  
                     20                                    25                                    30

Ile Lys Glu Pro Arg Arg Arg Arg Pro Met Leu Leu Ala Glu Val Pro  
                     35                                    40                                    45

Cys Leu Xaa Glu Gly Ala Arg Arg Thr Gly Phe Arg Gln Arg Gln Ala  
                     50                                    55                                    60

Leu Arg Ser Arg Leu Trp Pro Leu Ala Val Arg His Ala Cys Val Xaa  
                     65                                    70                                    75                                    80

Phe Lys Arg Asp Cys Gly Ser Arg Glu Arg Pro Leu Arg Leu Ser Glu  
                     85                                    90                                    95

Val Gly Ser Ser Arg Ala Gly Ser Glu Ser Cys Ser Xaa Gly Ser Thr  
                     100                                    105                                    110

Ser Leu Asp Ala His Pro  
                     115

<210> 59

<211> 491

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(102)

&lt;400&gt; 59

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cgg cgg tat tat agg aca cgg ccg cct gct ggt aac atc tgt aat tta      48
Arg Arg Tyr Tyr Arg Thr Arg Pro Pro Ala Gly Asn Ile Cys Asn Leu
  1             5             10             15

tca ttg tat ccc gtc gtc ccg tgt tcc aaa ctg gga atc ttt tct ttc      96
Ser Leu Tyr Pro Val Val Pro Cys Ser Lys Leu Gly Ile Phe Ser Phe
      20             25             30

ctg agc tgacggtttg gcccgcaagc tcagccgagt acgaaaccat gattaggttg      152
Leu Ser

gaggcctaatt gtgctttttc gccagctgtc aaacgggcag ccaaggttga tttctctatg 212

agttgtcctc cgcgctctcg aattggtatt tcgtggtttc agattgaaag cgtcactcga 272

gctattacga ggcgtttcag caaaaaggaa gaatcactca gacacctgac cgacgcttga 332

tgtgctggcg gttgtgcaaa tccaggcatc actcaacgcc gatgctcagc aggacctatg 392

gatcttaaga ggttctgttc cactacatca gtgagagttt caaaaagaat cctgataact 452

acgcgcttct acaggtgccg cctttatggc aacgatccg                        491

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&lt;210&gt; 60

&lt;211&gt; 34

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 60

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Arg Arg Tyr Tyr Arg Thr Arg Pro Pro Ala Gly Asn Ile Cys Asn Leu
  1             5             10             15

Ser Leu Tyr Pro Val Val Pro Cys Ser Lys Leu Gly Ile Phe Ser Phe
      20             25             30

Leu Ser

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<210> 61  
 <211> 387  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <221> CDS  
 <222> (1)..(387)

<400> 61  
 cgg atc gct ctg agt ctc ttt ggg ctc cct gcc gca tgc agg cat gaa 48  
 Arg Ile Ala Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu  
     1                    5                    10                    15

agt gtc tcg ccg cga gag aca gag aag gaa gtg cag agc gag cgt ggg 96  
 Ser Val Ser Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly  
                     20                    25                    30

cga gaa cgg acg cag aaa ggc gca ggc gag aag gag acc ggc gta gac 144  
 Arg Glu Arg Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp  
             35                    40                    45

gga gtg act gga gag cag gtc tta gcg ctc act aag ggt gaa cct gaa 192  
 Gly Val Thr Gly Glu Gln Val Leu Ala Leu Thr Lys Gly Glu Pro Glu  
         50                    55                    60

gcg gca gaa gaa gcg aga gaa gag gac gag gga aag gga gaa gac aga 240  
 Ala Ala Glu Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg  
     65                    70                    75                    80

tgg tac gag gaa ggc gcg agg cga gag aaa gag gcg gct cga gtc atg 288  
 Trp Tyr Glu Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met  
             85                    90                    95

tcc act ccg cag acg tat gcc gaa gcc acc gac aca aca gct gca tgc 336  
 Ser Thr Pro Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala Ala Cys  
             100                    105                    110

aga gac gaa agg gag ctc gcc tcg ggg gtc gaa gag aag aca cag gat 384  
 Arg Asp Glu Arg Glu Leu Ala Ser Gly Val Glu Glu Lys Thr Gln Asp  
         115                    120                    125

ccg 387  
 Pro

<210> 62  
 <211> 129

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 62

Arg Ile Ala Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu  
 1 5 10 15

Ser Val Ser Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly  
 20 25 30

Arg Glu Arg Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp  
 35 40 45

Gly Val Thr Gly Glu Gln Val Leu Ala Leu Thr Lys Gly Glu Pro Glu  
 50 55 60

Ala Ala Glu Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg  
 65 70 75 80

Trp Tyr Glu Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met  
 85 90 95

Ser Thr Pro Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala Ala Cys  
 100 105 110

Arg Asp Glu Arg Glu Leu Ala Ser Gly Val Glu Glu Lys Thr Gln Asp  
 115 120 125

Pro

&lt;210&gt; 63

&lt;211&gt; 417

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;223&gt; N = unknown at 72, 74, 139 and 141

&lt;220&gt;

&lt;223&gt; At locations 25 and 47, Xaa = unknown

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(417)

&lt;223&gt; Xaa = unknown at 25 and 47

&lt;400&gt; 63

ctt gca tgc gct gtg gca atg gaa gaa gca ccc gcg cca ggg caa cca 48  
 Leu Ala Cys Ala Val Ala Met Glu Glu Ala Pro Ala Pro Gly Gln Pro  
 1 5 10 15

ccc gaa gaa ggg gac gat ggc ggn tnt cag cag cgc ctg gag atc gct 96  
 Pro Glu Glu Gly Asp Asp Gly Xaa Xaa Gln Gln Arg Leu Glu Ile Ala  
 20 25 30

ctg agt ctc ttt ggg ctc cct gcc gca tgc agg cat gaa agt ntn tcg 144  
 Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu Ser Xaa Ser  
 35 40 45

ccg cga gag aca gag aag gaa gtg cag agc gag cgt ggg cga gaa cgg 192  
 Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly Arg Glu Arg  
 50 55 60

acg cag aaa ggc gca ggc gag aag gag acc ggc gta gac gga gtg act 240  
 Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp Gly Val Thr  
 65 70 75 80

gga gag cag ctc tta gcg ctc act aag ggt gaa cct gaa gcg gca gaa 288  
 Gly Glu Gln Leu Leu Ala Leu Thr Lys Gly Glu Pro Glu Ala Ala Glu  
 85 90 95

gaa gcg aga gaa gag gac gag gga aag gga gaa gac aga tgg aac gag 336  
 Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg Trp Asn Glu  
 100 105 110

gaa ggc gcg agg cga gag aaa gag gcg gct cga gtc atg tcc act ccg 384  
 Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met Ser Thr Pro  
 115 120 125

cag acg tat gcc gaa gcc acc gac aca aca gcg 417  
 Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala  
 130 135

&lt;210&gt; 64

&lt;211&gt; 139

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 64

Leu Ala Cys Ala Val Ala Met Glu Glu Ala Pro Ala Pro Gly Gln Pro  
 1 5 10 15

Pro Glu Glu Gly Asp Asp Gly Xaa Xaa Gln Gln Arg Leu Glu Ile Ala

20 25 30  
 Leu Ser Leu Phe Gly Leu Pro Ala Ala Cys Arg His Glu Ser Xaa Ser  
 35 40 45  
 Pro Arg Glu Thr Glu Lys Glu Val Gln Ser Glu Arg Gly Arg Glu Arg  
 50 55 60  
 Thr Gln Lys Gly Ala Gly Glu Lys Glu Thr Gly Val Asp Gly Val Thr  
 65 70 75 80  
 Gly Glu Gln Leu Leu Ala Leu Thr Lys Gly Glu Pro Glu Ala Ala Glu  
 85 90 95  
 Glu Ala Arg Glu Glu Asp Glu Gly Lys Gly Glu Asp Arg Trp Asn Glu  
 100 105 110  
 Glu Gly Ala Arg Arg Glu Lys Glu Ala Ala Arg Val Met Ser Thr Pro  
 115 120 125  
 Gln Thr Tyr Ala Glu Ala Thr Asp Thr Thr Ala  
 130 135

&lt;210&gt; 65

&lt;211&gt; 416

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;223&gt; N = unknown at 74 and 107

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(414)

&lt;400&gt; 65

ccg gat cgc ggg aga gaa gaa cgt gag gga gaa gaa gag agt gcc gag 48  
 Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Glu Glu Ser Ala Glu  
 1 5 10 15  
 gct ttg cca gac cat aag cgg ggg cca gga aaa gag ctg gag gaa ggc 96  
 Ala Leu Pro Asp His Lys Arg Gly Pro Gly Lys Glu Leu Glu Glu Gly  
 20 25 30  
 cga gac tcg cag gtc cgt ggt gag gag agc ggg cgc agc tcg ctt tcg 144  
 Arg Asp Ser Gln Val Arg Gly Glu Glu Ser Gly Arg Ser Ser Leu Ser  
 35 40 45

cag gag agg gaa agt ttt cgt tct cag cgn gtc tcg gct gag ggt cag 192  
 Gln Glu Arg Glu Ser Phe Arg Ser Gln Xaa Val Ser Ala Glu Gly Gln  
 50 55 60

gag gtg gag gca gcn tct gtc aag gcg ctt gaa gag gca aag tcg aac 240  
 Glu Val Glu Ala Xaa Ser Val Lys Ala Leu Glu Glu Ala Lys Ser Asn  
 65 70 75 80

gac aga ccc gac ggc gag agc aac gag ctg cgt cgc ttg tca ccc acc 288  
 Asp Arg Pro Asp Gly Glu Ser Asn Glu Leu Arg Arg Leu Ser Pro Thr  
 85 90 95

agc cag aca gag caa gaa ggc tcc gtc gag aaa gaa ggg aca tca gag 336  
 Ser Gln Thr Glu Gln Glu Gly Ser Val Glu Lys Glu Gly Thr Ser Glu  
 100 105 110

gcg acg atg aac gac caa gac gag aca ggg aag gaa aaa caa gac caa 384  
 Ala Thr Met Asn Asp Gln Asp Glu Thr Gly Lys Glu Lys Gln Asp Gln  
 115 120 125

cga gag gtg cct gtg ccc cgc gct ctt cgc tt 416  
 Arg Glu Val Pro Val Pro Arg Ala Leu Arg  
 130 135

&lt;210&gt; 66

&lt;211&gt; 138

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 66

Pro Asp Arg Gly Arg Glu Glu Arg Glu Gly Glu Glu Glu Ser Ala Glu  
 1 5 10 15

Ala Leu Pro Asp His Lys Arg Gly Pro Gly Lys Glu Leu Glu Glu Gly  
 20 25 30

Arg Asp Ser Gln Val Arg Gly Glu Glu Ser Gly Arg Ser Ser Leu Ser  
 35 40 45

Gln Glu Arg Glu Ser Phe Arg Ser Gln Xaa Val Ser Ala Glu Gly Gln  
 50 55 60

Glu Val Glu Ala Xaa Ser Val Lys Ala Leu Glu Glu Ala Lys Ser Asn  
 65 70 75 80

Asp Arg Pro Asp Gly Glu Ser Asn Glu Leu Arg Arg Leu Ser Pro Thr



85

90

95

Ser Gln Thr Glu Gln Glu Gly Ser Val Glu Lys Glu Gly Thr Ser Glu  
 100 105 110

Ala Thr Met Asn Asp Gln Asp Glu Thr Gly Lys Glu Lys Gln Asp Gln  
 115 120 125

Arg Glu Val Pro Val Pro Arg Ala Leu Arg  
 130 135

&lt;210&gt; 67

&lt;211&gt; 500

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 67

ccgagaatca tggtacgcca tgtagacagc gtttagggag tgcagacatt ttaatctgga 60  
 cggagtccaa gtggacgcgg atgtagatat ctgtcgagc acctccgcag ttgcgctagg 120  
 gattctgatg ctgctagttt taacatccaa aactctgact tcgcttggtg atctccaggt 180  
 gcatatacat gcgaaggcaa tcgtgtttgt gagaggcgaa tgtacgaatt tcagtgtctt 240  
 tgtgtggaag tcaagttccc ctgaaccagc tgcttgtttt attctaccgc taatgtatga 300  
 agcttagcct cgtgtcctct tcgcccgtac acgagacacg atccaagagt catacaaatt 360  
 cttgcggcgg tgaggttaatt gtcaacagaa acaaaagtcg cgggtatctg tgggtgtctct 420  
 gcttctgcac ttccaaggac cgccgcaagt tcggcccgat cggttggaac attcagtacg 480  
 agttcacgac ggaggatccg 500

&lt;210&gt; 68

&lt;211&gt; 321

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (219)

&lt;400&gt; 68

cgg cgg gac ttg cgg act tcg gtc tgg gac gct cgg gtg tac gta cac 48

Arg Arg Asp Leu Arg Thr Ser Val Trp Asp Ala Arg Val Tyr Val His  
 1 5 10 15  
 ctg gcg ggg ggc cag agg cgc tgc aac gag tcg cgg ggg atg gag gaa 96  
 Leu Ala Gly Gly Gln Arg Arg Cys Asn Glu Ser Arg Gly Met Glu Glu  
 20 25 30  
 gcg agg aaa agg agg tgt ctc gcg atg cgg tgc cag tgg act tcg tct 144  
 Ala Arg Lys Arg Arg Cys Leu Ala Met Arg Cys Gln Trp Thr Ser Ser  
 35 40 45  
 gcg cta gat tgg agg gag agc tgg aaa aat gcc gag aca gct tcg cac 192  
 Ala Leu Asp Trp Arg Glu Ser Trp Lys Asn Ala Glu Thr Ala Ser His  
 50 55 60  
 gtc aca ttc ccg acg aaa cgc ccg cca tgaaggaaat cacagacatc 239  
 Val Thr Phe Pro Thr Lys Arg Pro Pro  
 65 70  
 accaaccttc ccgcggtggc taaaggaccg tcctgtgtat gtacagtttt tccaggcgaa 299  
 agccgagaga cagcgaaacc gg 321

&lt;210&gt; 69

&lt;211&gt; 73

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 69

Arg Arg Asp Leu Arg Thr Ser Val Trp Asp Ala Arg Val Tyr Val His  
 1 5 10 15  
 Leu Ala Gly Gly Gln Arg Arg Cys Asn Glu Ser Arg Gly Met Glu Glu  
 20 25 30  
 Ala Arg Lys Arg Arg Cys Leu Ala Met Arg Cys Gln Trp Thr Ser Ser  
 35 40 45  
 Ala Leu Asp Trp Arg Glu Ser Trp Lys Asn Ala Glu Thr Ala Ser His  
 50 55 60  
 Val Thr Phe Pro Thr Lys Arg Pro Pro  
 65 70

&lt;210&gt; 70

&lt;211&gt; 513

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(513)

&lt;400&gt; 70

cg	g	a	t	c	a	g	c	t	t	a	t	c	c	a	c	t	g	c	c	c	c	c	g	a	a	g	a	c	t	t	g	a	c		48										
Arg	Asp	Gln	Ala	Ser	Met	Pro	Leu	Pro	Pro	Ala	Pro	Glu	Asp	Phe	Asp																														
1					5					10					15																														
c	t	g	c	c	t	a	t	g	c	c	a	c	t	g	c	c	c	a	a	g	a	c	c	c	a	a	g	a	c	t	t	g	a	c	c	a	g	c	t		96				
Leu	Pro	Pro	Met	Pro	Leu	Pro	Glu	Ala	Pro	Glu	Asp	Phe	Asp	Gln	Ala																														
			20						25					30																															
c	c	t	a	t	g	c	c	a	c	t	g	c	c	c	a	a	g	a	c	c	c	a	a	g	a	c	t	t	g	a	c	c	a	g	c	t	c	c	t	a	t	g		144	
Pro	Met	Pro	Leu	Pro	Glu	Ala	Pro	Glu	Asp	Phe	Asp	Gln	Ala	Pro	Met																														
			35					40					45																																
c	c	a	c	t	g	c	c	c	a	a	g	a	c	c	c	a	a	g	a	c	t	t	g	a	c	c	a	c	c	t	a	t	g	c	c	a	c	a	c	t	g		192		
Pro	Leu	Pro	Glu	Ala	Pro	Glu	Asp	Phe	Asp	Gln	Pro	Pro	Met	Pro	Leu																														
			50				55					60																																	
c	c	c	a	a	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	c	c	a	a	g	a	c	c	c	a	a		240	
Pro	Glu	Ala	Pro	Glu	Asp	Phe	Asp	Gln	Ala	Pro	Met	Pro	Leu	Pro	Glu																														
	65				70				75				80																																
g	c	a	c	c	a	a	g	t	c	t	t	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	a	g	a	c	c		288
Ala	Pro	Glu	Val	Phe	Asp	Gln	Ala	Pro	Met	Pro	Leu	Pro	Glu	Ala	Pro																														
				85					90				95																																
g	a	a	g	t	c	t	t	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	c		336		
Glu	Val	Phe	Asp	Gln	Ala	Pro	Met	Pro	Leu	Pro	Glu	Ala	Pro	Glu	Asp																														
			100					105				110																																	
t	t	t	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	a	g	a	c	c	a	a	g	a	c	c		384		
Phe	Asp	Gln	Ala	Pro	Met	Pro	Leu	Pro	Glu	Ala	Pro	Glu	Val	Phe	Asp																														
		115					120					125																																	
c	a	g	c	t	c	c	t	a	t	g	c	c	a	c	t	g	c	c	a	a	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	a	g	a	c	c		432		
Gln	Ala	Pro	Met	Pro	Leu	Pro	Glu	Ala	Pro	Glu	Asp	Phe	Asp	Gln	Ala																														
		130					135					140																																	
c	c	t	a	t	g	c	c	a	g	t	c	c	a	a	g	t	c	c	a	a	g	a	c	c	a	a	g	a	c	t	t	g	a	c	c	a	a	g	a	c	c		480		
Pro	Met	Pro	Val	Pro	Glu	Ala	Pro	Glu	Asp	Phe	Asp	Gln	Ala	Pro	Glu																														
	145					150				155			160																																

cca ctg ccc gag gca gcc gaa gaa ttt gat ccc  
 Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro  
 165 170

513

<210> 71  
 <211> 171  
 <212> PRT  
 <213> *Toxoplasma gondii*

<400> 71  
 Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp  
 1 5 10 15  
 Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala  
 20 25 30  
 Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met  
 35 40 45  
 Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu  
 50 55 60  
 Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu  
 65 70 75 80  
 Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro  
 85 90 95  
 Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp  
 100 105 110  
 Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp  
 115 120 125  
 Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala  
 130 135 140  
 Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu  
 145 150 155 160  
 Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro  
 165 170

<210> 72  
 <211> 528  
 <212> DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(528)

&lt;400&gt; 72

```

cga tct gaa cgt tgt gca acc gtt ggg gac cca ggt aca ggc gtc tcc 48
Arg Ser Glu Arg Cys Ala Thr Val Gly Asp Pro Gly Thr Gly Val Ser
  1             5             10             15

aac act gag gcg ggg gga aag cgc cca cac tgg cgt ctc agg cac ctt 96
Asn Thr Glu Ala Gly Gly Lys Arg Pro His Trp Arg Leu Arg His Leu
          20             25             30

caa tgc cac agg tat ccg gca tcc ttg gag aca gag ctt gag acg gag 144
Gln Cys His Arg Tyr Pro Ala Ser Leu Glu Thr Glu Leu Glu Thr Glu
          35             40             45

aca ctc gca cac aca ccc aga gag ctt gtg gtg aca aat cga acc ttc 192
Thr Leu Ala His Thr Pro Arg Glu Leu Val Val Thr Asn Arg Ser Leu
          50             55             60

ggg ttt gtc tcg ctt ctt cgc cag tcg ttc gcg tcg cag tca gaa gaa 240
Gly Phe Val Ser Leu Leu Arg Gln Ser Phe Ala Ser Gln Ser Glu Ala
          65             70             75             80

gtc aag gcg acc gcg gag acg ccg aca gag aca gag aca gtc ctt gtt 288
Val Lys Ala Thr Ala Glu Thr Pro Thr Glu Thr Glu Thr Val Leu Val
          85             90             95

gcg ggc gag cgc aac acc gcg aaa gaa aga gag aga aaa ggg cag gac 336
Ala Gly Glu Arg Asn Thr Ala Lys Glu Arg Glu Arg Lys Gly Gln Asp
          100             105             110

gaa gag gtt tcg cag aga gca gcg gag aac aag aga gga cga gta gaa 384
Glu Glu Val Ser Gln Arg Ala Ala Glu Asn Lys Arg Gly Arg Val Glu
          115             120             125

gac aca gac tac cgg gag acg gat aag aaa gcc gag aaa gat gtt gtt 432
Asp Thr Asp Tyr Arg Glu Thr Asp Lys Lys Ala Glu Lys Asp Glu Arg
          130             135             140

gaa gag aac ccc cga gga gac aca ggg gag cag aga agc gag aat gac 480
Glu Glu Asn Pro Arg Gly Asp Thr Gly Glu Gln Arg Ser Glu Lys His
          145             150             155             160

acg aga gat tta ttg gga cag gag aga gag aac gca tgg gag atc ccg 528

```

Thr Arg Asp Leu Leu Gly Gln Glu Arg Glu Asn Ala Trp Glu Ile Pro  
 165 170 175

<210> 73

<211> 176

<212> PRT

<213> *Toxoplasma gondii*

<400> 73

Arg Ser Glu Arg Cys Ala Thr Val Gly Asp Pro Gly Thr Gly Val Ser  
 1 5 10 15

Asn Thr Glu Ala Gly Gly Lys Arg Pro His Trp Arg Leu Arg His Leu  
 20 25 30

Gln Cys His Arg Tyr Pro Ala Ser Leu Glu Thr Glu Leu Glu Thr Glu  
 35 40 45

Thr Leu Ala His Thr Pro Arg Glu Leu Val Val Thr Asn Arg Ser Leu  
 50 55 60

Gly Phe Val Ser Leu Leu Arg Gln Ser Phe Ala Ser Gln Ser Glu Ala  
 65 70 75 80

Val Lys Ala Thr Ala Glu Thr Pro Thr Glu Thr Glu Thr Val Leu Val  
 85 90 95

Ala Gly Glu Arg Asn Thr Ala Lys Glu Arg Glu Arg Lys Gly Gln Asp  
 100 105 110

Glu Glu Val Ser Gln Arg Ala Ala Glu Asn Lys Arg Gly Arg Val Glu  
 115 120 125

Asp Thr Asp Tyr Arg Glu Thr Asp Lys Lys Ala Glu Lys Asp Glu Arg  
 130 135 140

Glu Glu Asn Pro Arg Gly Asp Thr Gly Glu Gln Arg Ser Glu Lys His  
 145 150 155 160

Thr Arg Asp Leu Leu Gly Gln Glu Arg Glu Asn Ala Trp Glu Ile Pro  
 165 170 175

<210> 74

<211> 375

<212> DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(375)

&lt;400&gt; 74

```

ccg gag gag tac aag tgc agc aaa acc acg tac gaa gac agc tgc acc 48
Pro Glu Glu Tyr Lys Cys Ser Lys Thr Thr Tyr Glu Asp Ser Cys Thr
  1             5             10             15

gat gtc gct gtc cag gtc ccc gac acc tgc tac cgc act gtc gat cag 96
Asp Val Ala Val Gln Val Pro Asp Thr Cys Tyr Arg Thr Val Asp Gln
      20             25             30

aag aag gct tac aag tgc aag aaa acg ctg acg aaa aac caa tgc acg 144
Lys Lys Ala Tyr Lys Cys Lys Lys Thr Leu Thr Lys Asn Gln Cys Thr
      35             40             45

aag gtt cca gtc cag gtt cca agc aca tgc acg aag acg gcg atg tca 192
Lys Val Pro Val Gln Val Pro Ser Thr Cys Thr Lys Thr Ala Met Ser
      50             55             60

aag gag gcg tac gac tgc tcg aag acc gag ttc cgc acc gag tgc acc 240
Lys Glu Ala Tyr Asp Cys Ser Lys Thr Glu Phe Arg Thr Glu Cys Thr
      65             70             75             80

gac gaa gtc gag caa gtc ccg tgc atg ggc aaa gag tgc aag ctg cgc 288
Asp Glu Val Glu Gln Val Pro Cys Met Gly Lys Glu Cys Lys Leu Arg
      85             90             95

cag ctg aag aag aag cgc gtc tgc agg cag gtc ccg ttc acc agc aag 336
Gln Leu Lys Lys Lys Arg Val Cys Arg Gln Val Pro Phe Thr Ser Lys
      100             105             110

aac gtc tgc tac aaa aat gtg ccc acg gag cag acg tcg 375
Asn Val Cys Tyr Lys Asn Val Pro Thr Glu Gln Thr Ser
      115             120             125

```

&lt;210&gt; 75

&lt;211&gt; 125

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 75

```

Pro Glu Glu Tyr Lys Cys Ser Lys Thr Thr Tyr Glu Asp Ser Cys Thr
  1             5             10             15

```

Asp Val Ala Val Gln Val Pro Asp Thr Cys Tyr Arg Thr Val Asp Gln  
 20 25 30

Lys Lys Ala Tyr Lys Cys Lys Lys Thr Leu Thr Lys Asn Gln Cys Thr  
 35 40 45

Lys Val Pro Val Gln Val Pro Ser Thr Cys Thr Lys Thr Ala Met Ser  
 50 55 60

Lys Glu Ala Tyr Asp Cys Ser Lys Thr Glu Phe Arg Thr Glu Cys Thr  
 65 70 75 80

Asp Glu Val Glu Gln Val Pro Cys Met Gly Lys Glu Cys Lys Leu Arg  
 85 90 95

Gln Leu Lys Lys Lys Arg Val Cys Arg Gln Val Pro Phe Thr Ser Lys  
 100 105 110

Asn Val Cys Tyr Lys Asn Val Pro Thr Glu Gln Thr Ser  
 115 120 125

<210> 76

<211> 543

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(267)

<400> 76

cga tcc aac agt tta cga ggt aca agg caa cag ccg aac ctc tac aaq 48  
 Arg Ser Asn Ser Leu Arg Gly Thr Arg Gln Gln Pro Asn Leu Tyr Glu  
 1 5 10 15

cac gtg tcc cca cgg ttc acg ctc tcc cat gga aaa gca aag coa ttc 96  
 His Val Ser Pro Arg Phe Thr Leu Ser His Gly Lys Ala Lys Arg Phe  
 20 25 30

ctc cat tat cac cac tgc cac tgc cat tcc agc cta aga atc cta cac 144  
 Leu His Tyr His His Cys His Cys His Ser Ser Leu Arg Ile Leu His  
 35 40 45

ttc aaa gac gaa ctt ttg cat cgt ccg tgc gtc tcc cgt ggc caa cac 192  
 Phe Lys Asp Glu Leu Leu His Arg Pro Cys Val Ser Arg Gly Gln His  
 50 55 60



cct caa gcc aaa aga gag ggc acc ttc tac act gcc cac gca atc acc 240  
 Pro Gln Ala Lys Arg Glu Gly Thr Phe Tyr Thr Ala His Ala Ile Thr  
 65 70 75 80

ctg tgc ggc ggc aca caa aag cga aac tgacacacgc tactgccgtt 287  
 Leu Cys Gly Gly Thr Gln Lys Arg Asn  
 85

ccggaaagtg gtctgaaaga aactgacaac agccgcaaag agacatttac ccggtgcctg 347  
 gcgtggtcaa aaatccggca taatggtttc tgcgcatacct ccattcagcc gcccaacatc 407  
 tgcggtcgtt cttccgtcga aactatgaca caacgagcct tgtggaacaa aacgggttcgt 467  
 actgacgaca ttgcctgggt cggattcact gcatgtttgc caggggtgcat ttccacgggtg 527  
 ctctgcgtcg atccccg 543

<210> 77

<211> 89

<212> PRT

<213> Toxoplasma gondii

<400> 77

Arg Ser Asn Ser Leu Arg Gly Thr Arg Gln Gln Pro Asn Leu Tyr Glu  
 1 5 10 15

His Val Ser Pro Arg Phe Thr Leu Ser His Gly Lys Ala Lys Arg Phe  
 20 25 30

Leu His Tyr His His Cys His Cys His Ser Ser Leu Arg Ile Leu His  
 35 40 45

Phe Lys Asp Glu Leu Leu His Arg Pro Cys Val Ser Arg Gly Gln His  
 50 55 60

Pro Gln Ala Lys Arg Glu Gly Thr Phe Tyr Thr Ala His Ala Ile Thr  
 65 70 75 80

Leu Cys Gly Gly Thr Gln Lys Arg Asn  
 85

<210> 78

<211> 573

<212> DNA

<213> Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(573)

&lt;400&gt; 78

```

ccg gcg tcg tcg agc tcg agg ctg ggc aag ctg gct tac gac gat gca 48
Pro Ala Ser Ser Ser Ser Arg Leu Gly Lys Leu Ala Tyr Asp Asp Ala
  1             5             10             15

gga ggt gga cga gga gcg agc tcg cca cca tct tct aag ttg ttt gtt 96
Gly Gly Gly Arg Gly Ala Ser Ser Pro Pro Ser Ser Lys Leu Phe Val
          20             25             30

tcc cca gtc aac gac agg tca cgg atg gca gat caa cga aaa cct gca 144
Ser Pro Val Asn Asp Arg Ser Arg Met Ala Asp Gln Arg Lys Pro Ala
          35             40             45

ccc gaa caa tcg tcc aat cac gat tcg gaa tgc tgt tgc cta cgc tgt 192
Pro Glu Gln Ser Ser Asn His Asp Ser Glu Cys Cys Cys Leu Arg Cys
          50             55             60

ctg agt gag aag acg ctg atg atg gca cag ctc tgc agg cct gca cct 240
Leu Ser Glu Lys Thr Leu Met Met Ala Gln Leu Cys Arg Pro Ala Pro
          65             70             75             80

gta acc ctg tct gta aca gag agg aac cta ttt gga gat aat ggc aga 288
Val Thr Leu Ser Val Thr Glu Arg Asn Leu Phe Gly Asp Asn Gly Arg
          85             90             95

gac gtc gtt gaa tgg gag ggt tca tgc gga ttt ttt tct gga aat gca 336
Asp Val Val Glu Trp Glu Gly Ser Cys Gly Phe Phe Ser Gly Asn Ala
          100             105             110

tcg act agg cca tct ctg cag ttc tcc cct cac cgt gtc atc gat gcc 384
Ser Thr Arg Pro Ser Leu Gln Phe Ser Pro His Arg Val Ile Asp Ala
          115             120             125

cca aca gcc aat gac gat atg aga gat tgc aga gca gcc cct gaa gac 432
Pro Thr Ala Asn Asp Asp Met Arg Asp Cys Arg Ala Ala Pro Glu Asp
          130             135             140

ggg aca ggt acc tca aag gca aat att cac cgc agt agc aac ata aca 480
Gly Thr Gly Thr Ser Lys Ala Asn Ile His Arg Ser Ser Asn Ile Thr
          145             150             155             160

aaa acg aag gaa gag aat ggt aga gat gtg tgt gag gga ctc agg aaa 528
Lys Thr Lys Glu Glu Asn Gly Arg Asp Val Cys Glu Gly Leu Arg Lys

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165

170

175

ccg ttg cag gac gat tct gaa gga gtc caa caa cct ctt ccg ccg 573  
 Pro Leu Gln Asp Asp Ser Glu Gly Val Gln Gln Pro Leu Pro Pro  
 180 185 190

&lt;210&gt; 79

&lt;211&gt; 191

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 79

Pro Ala Ser Ser Ser Ser Arg Leu Gly Lys Leu Ala Tyr Asp Asp Ala  
 1 5 10 15

Gly Gly Gly Arg Gly Ala Ser Ser Pro Pro Ser Ser Lys Leu Phe Val  
 20 25 30

Ser Pro Val Asn Asp Arg Ser Arg Met Ala Asp Gln Arg Lys Pro Ala  
 35 40 45

Pro Glu Gln Ser Ser Asn His Asp Ser Glu Cys Cys Cys Leu Arg Cys  
 50 55 60

Leu Ser Glu Lys Thr Leu Met Met Ala Gln Leu Cys Arg Pro Ala Pro  
 65 70 75 80

Val Thr Leu Ser Val Thr Glu Arg Asn Leu Phe Gly Asp Asn Gly Arg  
 85 90 95

Asp Val Val Glu Trp Glu Gly Ser Cys Gly Phe Phe Ser Gly Asn Ala  
 100 105 110

Ser Thr Arg Pro Ser Leu Gln Phe Ser Pro His Arg Val Ile Asp Ala  
 115 120 125

Pro Thr Ala Asn Asp Asp Met Arg Asp Cys Arg Ala Ala Pro Glu Asp  
 130 135 140

Gly Thr Gly Thr Ser Lys Ala Asn Ile His Arg Ser Ser Asn Ile Thr  
 145 150 155 160

Lys Thr Lys Glu Glu Asn Gly Arg Asp Val Cys Glu Gly Leu Arg Lys  
 165 170 175

Pro Leu Gln Asp Asp Ser Glu Gly Val Gln Gln Pro Leu Pro Pro  
 180 185 190

<210> 80  
 <211> 1835  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <221> CDS  
 <222> (1)..(1833)

<400> 80

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cgg atc agt ggg gac cag tac tct tgt ctt caa cga gga gcg gga gga      48
Arg Ile Ser Gly Asp Gln Tyr Ser Cys Leu Gln Arg Gly Ala Gly Gly
   1             5             10             15

gac aag gag aca gca acc gag aga gaa gag agg aac aga gaa gat gcg      96
Asp Lys Glu Thr Ala Thr Glu Arg Glu Glu Arg Asn Arg Glu Asp Ala
           20             25             30

ccc tcc ttt ctt gaa gga gga ctc gga gat gac gag aca gag aga gcg      144
Pro Ser Phe Leu Glu Gly Gly Leu Gly Asp Asp Glu Thr Glu Arg Ala
           35             40             45

aag caa gcg agt gag ttg ccc gcg tct ctt tgc tct ttc gcc gca gca      192
Lys Gln Ala Ser Glu Leu Pro Ala Ser Leu Cys Ser Phe Ala Ala Ala
           50             55             60

cgc agg ggc gcg agc cgc gca gag aag aca ggc gca aag ggg gag gaa      240
Arg Arg Gly Ala Ser Arg Ala Glu Lys Thr Gly Ala Lys Gly Glu Glu
           65             70             75             80

gcc aga gag aaa gaa gtc agt ttc ggt gaa gac agt ggg cta tcc aga      288
Ala Arg Glu Lys Glu Val Ser Phe Gly Glu Asp Ser Gly Leu Ser Arg
           85             90             95

cag gtg gac atg gac agt tcg cag gaa tct gtc aac gaa gga gag ccg      336
Gln Val Asp Met Asp Ser Ser Gln Glu Ser Val Asn Glu Gly Glu Pro
          100             105             110

cta cac gac aga gcc gca ggg gag gac gca gaa ggc ggg gga gca gag      384
Leu His Asp Arg Ala Ala Gly Glu Asp Ala Glu Gly Gly Gly Ala Glu
          115             120             125

gcg aac gac gga gac aga gag gga gac gag aag gag act cga gac gtc      432
Ala Asn Asp Gly Asp Arg Glu Gly Asp Glu Lys Glu Thr Arg Asp Val
          130             135             140

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gag gac gaa gga gag acg cgt cgt tct tcc tct ttc gct gaa caa act	480
Glu Asp Glu Gly Glu Thr Arg Arg Ser Ser Phe Ala Glu Gln Thr	
145 150 155 160	
gga aat gaa aga acc gag atg aga acc aga cat ggg ggt gac gag ggc	528
Gly Asn Glu Arg Thr Glu Met Arg Thr Arg His Gly Gly Asp Glu Gly	
165 170 175	
tgg acc tcg aag tcg aat cgg ttc gct ttt gcc tgc cct cgg ttt tcc	576
Trp Thr Ser Lys Ser Asn Arg Phe Ala Phe Ala Cys Pro Arg Phe Ser	
180 185 190	
aaa tct gat gtc tgc tgt tct ccc cag gct cgg ctg tct ttg cct gaa	624
Lys Ser Asp Val Cys Cys Ser Pro Gln Ala Arg Leu Ser Leu Pro Glu	
195 200 205	
cag tcc cta ggc tcc tct ccg tcg tcg ccc att tct gtc aca aat gat	672
Gln Ser Leu Gly Ser Ser Pro Ser Ser Pro Ile Ser Val Thr Asn Asp	
210 215 220	
gtc tat gct ctc ttc gat tcg tct gca tct cct ctg cat gcg gga gag	720
Val Tyr Ala Leu Phe Asp Ser Ser Ala Ser Pro Leu His Ala Gly Glu	
225 230 235 240	
tta tct tct ctt ccc ggc gcg gtc tcg gcc tca gag cgc cta ttg act	768
Leu Ser Ser Leu Pro Gly Ala Val Ser Ala Ser Glu Arg Leu Leu Thr	
245 250 255	
gct ccg gca gaa ata ggt ccc tcg gcc tcc tca gcc tgc ctc tcc gtt	816
Ala Pro Ala Glu Ile Gly Pro Ser Ala Ser Ser Ala Cys Leu Ser Val	
260 265 270	
tct tgt ggt cca ggc gaa atg tct ccg aca gcg gat acg acg aga cac	864
Ser Cys Gly Pro Gly Glu Met Ser Pro Thr Ala Asp Thr Thr Arg His	
275 280 285	
gac gcg gaa gag aga gaa cgc agg aga gcg gag gaa gag aag gag aga	912
Asp Ala Glu Glu Arg Glu Arg Arg Arg Ala Glu Glu Glu Lys Glu Arg	
290 295 300	
gag aga cag gaa gaa gaa gag aga gaa cgc agg aga gtg gag gaa gag	960
Glu Arg Gln Glu Glu Glu Glu Arg Glu Arg Arg Val Glu Glu Glu	
305 310 315 320	
aag gag aga gag aga cag gaa gaa gaa gag aga gaa cgc agg aga gtg	1008
Lys Glu Arg Glu Arg Gln Glu Glu Glu Glu Arg Glu Arg Arg Arg Val	
325 330 335	

gag gaa gag aag gcg aga cag aga gag gaa gat gag aga gaa cgc agg	1056
Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Asp Glu Arg Glu Arg Arg	
340 345 350	
aga gtg gag gaa gag aag gcg aga cag aga gag gaa gaa gag aga gaa	1104
Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu	
355 360 365	
cgc agg aga gtg gag gaa gag aag gcg aga cag aga gag gaa gaa gaa	1152
Arg Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu	
370 375 380	
gag aga gaa cgc agg aga gtg gag gaa gag aag gcg aga cag aga gag	1200
Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu	
385 390 395 400	
gaa gaa gaa gag aga gaa cgc agg aga gtg gag gaa gag aag gcg aga	1248
Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg	
405 410 415	
cag aga gag gaa gaa gaa gag aga gaa ggc agg aga gtg gag gaa gag	1296
Gln Arg Glu Glu Glu Glu Glu Arg Glu Gly Arg Arg Val Glu Glu Glu	
420 425 430	
aag gcg aga cag aga gag gaa gaa gaa gag aga gaa ggc agg aga gtg	1344
Lys Ala Arg Gln Arg Glu Glu Glu Glu Glu Arg Glu Gly Arg Arg Val	
435 440 445	
gag gaa gag aag gcg aga cag aga gag gaa gaa gag aga gaa cgc agg	1392
Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg	
450 455 460	
aga gta gag gaa gag aag gag aga gag aga cag gag gaa gag aga gaa	1440
Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Arg Glu	
465 470 475 480	
cgc agg aga gta gag gaa gag aag gag aga gag aga cag gag gaa gaa	1488
Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu	
485 490 495	
gag aga gaa cgc agg aga gtg gag gaa gag aag gag aga gag aga cag	1536
Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln	
500 505 510	
gaa gaa gaa aag aga gaa cgc agg aga gtg gag gaa gag aag gcg aga	1584
Glu Glu Glu Lys Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg	
515 520 525	

cag aga cag gaa gaa gaa ggg aga gaa aga caa aga gga gag gag aga 1632  
 Gln Arg Gln Glu Glu Glu Gly Arg Glu Arg Gln Arg Gly Glu Glu Arg  
 530 535 540

gaa gag aga gag aga gaa ttt caa cag cgc gag cgg gag ctg aag aca 1680  
 Glu Glu Arg Glu Arg Glu Phe Gln Gln Arg Glu Arg Glu Leu Lys Thr  
 545 550 555 560

cgg cta gta gag ctt cag aga gag cac gca gag tct gtt gaa acg tgg 1728  
 Arg Leu Val Glu Leu Gln Arg Glu His Ala Glu Ser Val Glu Thr Trp  
 565 570 575

atg aag gag caa gga gaa cga gaa agg cac ttg act cag gat tgg gag 1776  
 Met Lys Glu Gln Gly Glu Arg Glu Arg His Leu Thr Gln Asp Trp Glu  
 580 585 590

agg aaa ttg cat gcg ttt gaa gag cag agt cgg act gtg ttg ctc caa 1824  
 Arg Lys Leu His Ala Phe Glu Glu Gln Ser Arg Thr Val Leu Leu Gln  
 595 600 605

gag aga tcc cg 1835  
 Glu Arg Ser  
 610

<210> 81

<211> 611

<212> PRT

<213> *Toxoplasma gondii*

<400> 81

Arg Ile Ser Gly Asp Gln Tyr Ser Cys Leu Gln Arg Gly Ala Gly Gly  
 1 5 10 15

Asp Lys Glu Thr Ala Thr Glu Arg Glu Glu Arg Asn Arg Glu Asp Ala  
 20 25 30

Pro Ser Phe Leu Glu Gly Gly Leu Gly Asp Asp Glu Thr Glu Arg Ala  
 35 40 45

Lys Gln Ala Ser Glu Leu Pro Ala Ser Leu Cys Ser Phe Ala Ala Ala  
 50 55 60

Arg Arg Gly Ala Ser Arg Ala Glu Lys Thr Gly Ala Lys Gly Glu Glu  
 65 70 75 80

Ala Arg Glu Lys Glu Val Ser Phe Gly Glu Asp Ser Gly Leu Ser Arg  
 85 90 95

Gln Val Asp Met Asp Ser Ser Gln Glu Ser Val Asn Glu Gly Glu Pro  
100 105 110

Leu His Asp Arg Ala Ala Gly Glu Asp Ala Glu Gly Gly Gly Ala Glu  
115 120 125

Ala Asn Asp Gly Asp Arg Glu Gly Asp Glu Lys Glu Thr Arg Asp Val  
130 135 140

Glu Asp Glu Gly Glu Thr Arg Arg Ser Ser Ser Phe Ala Glu Gln Thr  
145 150 155 160

Gly Asn Glu Arg Thr Glu Met Arg Thr Arg His Gly Gly Asp Glu Gly  
165 170 175

Trp Thr Ser Lys Ser Asn Arg Phe Ala Phe Ala Cys Pro Arg Phe Ser  
180 185 190

Lys Ser Asp Val Cys Cys Ser Pro Gln Ala Arg Leu Ser Leu Pro Glu  
195 200 205

Gln Ser Leu Gly Ser Ser Pro Ser Ser Pro Ile Ser Val Thr Asn Asp  
210 215 220

Val Tyr Ala Leu Phe Asp Ser Ser Ala Ser Pro Leu His Ala Gly Glu  
225 230 235 240

Leu Ser Ser Leu Pro Gly Ala Val Ser Ala Ser Glu Arg Leu Leu Thr  
245 250 255

Ala Pro Ala Glu Ile Gly Pro Ser Ala Ser Ser Ala Cys Leu Ser Val  
260 265 270

Ser Cys Gly Pro Gly Glu Met Ser Pro Thr Ala Asp Thr Thr Arg His  
275 280 285

Asp Ala Glu Glu Arg Glu Arg Arg Arg Ala Glu Glu Glu Lys Glu Arg  
290 295 300

Glu Arg Gln Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu  
305 310 315 320

Lys Glu Arg Glu Arg Gln Glu Glu Glu Glu Arg Glu Arg Arg Arg Val  
325 330 335

Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Asp Glu Arg Glu Arg Arg  
340 345 350



Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu  
355 360 365

Arg Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu  
370 375 380

Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg Gln Arg Glu  
385 390 395 400

Glu Glu Glu Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg  
405 410 415

Gln Arg Glu Glu Glu Glu Arg Glu Gly Arg Arg Val Glu Glu Glu  
420 425 430

Lys Ala Arg Gln Arg Glu Glu Glu Glu Glu Arg Glu Gly Arg Arg Val  
435 440 445

Glu Glu Glu Lys Ala Arg Gln Arg Glu Glu Glu Glu Arg Glu Arg Arg  
450 455 460

Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu Arg Glu  
465 470 475 480

Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Gln Glu Glu Glu  
485 490 495

Glu Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Glu Arg Glu Arg Glu  
500 505 510

Glu Glu Glu Lys Arg Glu Arg Arg Arg Val Glu Glu Glu Lys Ala Arg  
515 520 525

Gln Arg Gln Glu Glu Glu Gly Arg Glu Arg Gln Arg Gly Glu Glu Arg  
530 535 540

Glu Glu Arg Glu Arg Glu Phe Gln Gln Arg Glu Arg Glu Leu Lys Thr  
545 550 555 560

Arg Leu Val Glu Leu Gln Arg Glu His Ala Glu Ser Val Glu Thr Trp  
565 570 575

Met Lys Glu Gln Gly Glu Arg Glu Arg His Leu Thr Gln Asp Trp Glu  
580 585 590

Arg Lys Leu His Ala Phe Glu Glu Gln Ser Arg Thr Val Leu Leu Gln  
595 600 605

Glu Arg Ser  
610

<210> 82

<211> 604

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(336)

<400> 82

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ccg atg caa ttt gtc tct cct tcc cct ttt gtg caa tcc gac tcc ccc 48
Pro Met Gln Phe Val Ser Pro Ser Pro Phe Val Gln Ser Asp Ser Pro
  1             5             10             15

tct tcg ccc ttc gca caa tcg gct tca cct cct cct tcc gag tac caa 96
Ser Ser Pro Phe Ala Gln Ser Ala Ser Pro Pro Pro Ser Glu Tyr Gln
      20             25             30

gac tct ctt tcc ctt cct ttg gca gaa tcc gtc tcg tcg ctt cct ttg 144
Asp Ser Leu Ser Leu Pro Leu Ala Glu Ser Val Ser Ser Leu Pro Leu
      35             40             45

gcg aaa cag gct tct cct ctt cac ttg aca caa cac cct tct ccc ctt 192
Ala Lys Gln Ala Ser Pro Leu His Leu Thr Gln His Pro Ser Pro Leu
      50             55             60

cta tgg aca cag cgg gcc tct cca tct cct ttc ttg gtt caa cgg gat 240
Leu Trp Thr Gln Arg Ala Ser Pro Ser Pro Phe Leu Val Gln Arg Asp
      65             70             75             80

tcg tca cct cct tct gcg tca atg cgg ctt tct gct cgt cct ttg gca 288
Ser Ser Pro Pro Ser Ala Ser Met Arg Leu Ser Ala Arg Pro Leu Ala
      85             90             95

aaa cat gtc tct ccc ctt ctg cgg gca aaa cag gct tct cct ttt cca 336
Lys His Val Ser Pro Leu Leu Arg Ala Lys Gln Ala Ser Pro Phe Pro
      100            105            110

tagaaccagc agcgggacctc tccatctcct ttcttggtcc accgggtttc gttctccttt 396

catccgtcaa tgcaggtttc atctcgctct ttggggaaac atgtccctcc ccttctccgg 456

gcaaaacagg cttctccttt tccatagaac cagcagcggg cctctccatc tccgttggtg 516

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gtccaccggg ttctgttctc ttttcatctg tcaatgcagg ttctgtctcg tgctttggca 576  
 aaacatgtcc ctccccttct ccgggggtg 604

<210> 83  
 <211> 112  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 83  
 Pro Met Gln Phe Val Ser Pro Ser Pro Phe Val Gln Ser Asp Ser Pro  
 1 5 10 15  
 Ser Ser Pro Phe Ala Gln Ser Ala Ser Pro Pro Pro Ser Glu Tyr Gln  
 20 25 30  
 Asp Ser Leu Ser Leu Pro Leu Ala Glu Ser Val Ser Ser Leu Pro Leu  
 35 40 45  
 Ala Lys Gln Ala Ser Pro Leu His Leu Thr Gln His Pro Ser Pro Leu  
 50 55 60  
 Leu Trp Thr Gln Arg Ala Ser Pro Ser Pro Phe Leu Val Gln Arg Asp  
 65 70 75 80  
 Ser Ser Pro Pro Ser Ala Ser Met Arg Leu Ser Ala Arg Pro Leu Ala  
 85 90 95  
 Lys His Val Ser Pro Leu Leu Arg Ala Lys Gln Ala Ser Pro Phe Pro  
 100 105 110

<210> 84  
 <211> 549  
 <212> DNA  
 <213> Toxoplasma gondii

<400> 84  
 ggcctttcca tcgccattct tgggtccaccg gggtccgtca tctcttcctc cgtcaaggca 60  
 ggtttgtctc ggccttgggc aaaaaatgtc cttccccttc tccgggcaac acaagcttgt 120  
 ctttttccat agaaccagca gcgggctttt catctcccgt tgggtgtcca ccgggtttcg 180  
 ttctcttttc atccgtcaat gcaggtttcg tctcgtcctt aggcaaaaca tgtctctccc 240

cttctccggg caaaacaagc ttgtcctttc ccatagaacc agcagcgggc ctctccatcg 300  
 ccattcttgg tccaccgggt ttctgtctct tttcatccgt caatgcaggt ttcgtctcgt 360  
 cctttggcaa aacatgtctc tccccttctc cgggcaaaac aggtttctcc ttttccatag 420  
 aaccagcagc gggcctctcc atctcctttc ttgggtccacc ggggttcggt ctcttttcat 480  
 ccgtcaatgc aggtttcgtc tcgtccttag gcaaaacatg tctctccctt tctccgggca 540  
 acacaagcg 549

&lt;210&gt; 85

&lt;211&gt; 270

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(270)

&lt;400&gt; 85

cgg acg gat gaa cac tgg tgc atc atg aag gat att ggc tac aag ggc 48  
 Arg Thr Asp Glu His Trp Cys Ile Met Lys Asp Ile Gly Tyr Lys Gly  
 1 5 10 15

aca gac tcg aag tca aca aaa gca aac tca gcg gca gag tgc caa caa gca 96  
 Thr Asp Ser Lys Ser Thr Lys Ala Asn Ser Ala Ala Glu Cys Gln Gln  
 20 25 30

atg tgc ctc aac gat gag agg tgt gac ttt ttc acg tgg caa caa gca 144  
 Met Cys Leu Asn Asp Glu Arg Cys Asp Phe Phe Thr Trp Gln Gln Ala  
 35 40 45

ggc aag cat tgt tgg ttt aag gct ggg gcg tcc act gcc tca aca aca 192  
 Gly Lys His Cys Trp Phe Lys Ala Gly Ala Ser Thr Ala Ser Thr Lys  
 50 55 60

tac aat cgg gct ggc gac tat tct gca cca aaa cac tgc ggc cta cca 240  
 Tyr Asn Arg Ala Gly Asp Tyr Ser Ala Pro Lys His Cys Gly Leu Ile  
 65 70 75 80

acc aca tgt gtc aag gag cgg acc aag tcg 270  
 Thr Thr Cys Val Lys Glu Arg Thr Lys Ser  
 85 90

<210> 86  
 <211> 90  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 86

Arg Thr Asp Glu His Trp Cys Ile Met Lys Asp Ile Gly Tyr Lys Gly  
 1 5 10 15

Thr Asp Ser Lys Ser Thr Lys Ala Asn Ser Ala Ala Glu Cys Gln Gln  
 20 25 30

Met Cys Leu Asn Asp Glu Arg Cys Asp Phe Phe Thr Trp Gln Gln Ala  
 35 40 45

Gly Lys His Cys Trp Phe Lys Ala Gly Ala Ser Thr Ala Ser Thr Lys  
 50 55 60

Tyr Asn Arg Ala Gly Asp Tyr Ser Ala Pro Lys His Cys Gly Leu Pro  
 65 70 75 80

Thr Thr Cys Val Lys Glu Arg Thr Lys Ser  
 85 90

<210> 87  
 <211> 306  
 <212> DNA  
 <213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(306)

<400> 87

cgg cgg caa caa atg ggc cct gtt cga gcc cct gac ctc caa ttc aac 48  
 Arg Arg Gln Gln Met Gly Pro Val Arg Ala Pro Asp Leu Gln Phe Asn  
 1 5 10 15

cag tcg cca ctg ctc ccc cac aac ctc ggc cct gcc cac gtt ccc atg 96  
 Gln Ser Pro Leu Leu Pro His Asn Leu Gly Pro Ala His Val Pro Met  
 20 25 30

gga ggt ctc ccg tcg cat cct cat atc tcg gac ttt cat aac tca tcg 144  
 Gly Gly Leu Pro Ser His Pro His Ile Ser Asp Phe His Asn Ser Ser  
 35 40 45

gag tcg cgc ccg caa cat ccg ctg ctt gcc agc ggg ctc gca tcg aga 192

Glu Ser Arg Pro Gln His Pro Leu Leu Ala Ser Gly Leu Ala Ser Arg  
 50 55 60  
 ctc gga cag ggc ctg acg ccc cag gag aga cag ttc gtg ctc tct caa 240  
 Leu Gly Gln Gly Leu Thr Pro Gln Glu Arg Gln Phe Val Leu Ser Gln  
 65 70 75 80  
 cag tct ggc gga tcg acc tcg ttc ctg ctg cct gcg ttg ccg tct ctc 288  
 Gln Ser Gly Gly Ser Thr Ser Phe Leu Leu Pro Ala Leu Pro Ser Leu  
 85 90 95  
 tca gag aac ctc tcc gcg 306  
 Ser Glu Asn Leu Ser Ala  
 100

<210> 88  
 <211> 102  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 88  
 Arg Arg Gln Gln Met Gly Pro Val Arg Ala Pro Asp Leu Gln Phe Asn  
 1 5 10 15  
 Gln Ser Pro Leu Leu Pro His Asn Leu Gly Pro Ala His Val Pro Met  
 20 25 30  
 Gly Gly Leu Pro Ser His Pro His Ile Ser Asp Phe His Asn Ser Ser  
 35 40 45  
 Glu Ser Arg Pro Gln His Pro Leu Leu Ala Ser Gly Leu Ala Ser Arg  
 50 55 60  
 Leu Gly Gln Gly Leu Thr Pro Gln Glu Arg Gln Phe Val Leu Ser Gln  
 65 70 75 80  
 Gln Ser Gly Gly Ser Thr Ser Phe Leu Leu Pro Ala Leu Pro Ser Leu  
 85 90 95  
 Ser Glu Asn Leu Ser Ala  
 100

<210> 89  
 <211> 804  
 <212> DNA  
 <213> Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(804)

&lt;400&gt; 89

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cgc gga ggc att tca gtt ccc aca ctt tcc atc atg aat cag agc acc 48
Arg Gly Gly Ile Ser Val Pro Thr Leu Ser Ile Met Asn Gln Ser Thr
  1             5             10             15

att gtt gcg acg tct gtg gtc gct ccg cag agc gca gtc tca ctt tcg 96
Ile Val Ala Thr Ser Val Val Ala Pro Gln Ser Ala Val Ser Leu Ser
      20             25             30

agg gcc cct agc cga cca ggg cct agc gag agt ttc ggt aaa cag caa 144
Arg Ala Pro Ser Arg Pro Gly Pro Ser Glu Ser Phe Gly Lys Gln Gln
      35             40             45

gaa agt cgt cca ggt gtt tcg ggt gct ggc ctc gct gaa agc aaa cgc 192
Glu Ser Arg Pro Gly Val Ser Gly Ala Gly Leu Ala Glu Ser Lys Arg
      50             55             60

gtg ccc agc ctt act cag ccg tct ctg gaa cgg tcc gta acc ata tca 240
Val Pro Ser Leu Thr Gln Pro Ser Leu Glu Arg Ser Val Thr Ile Ser
      65             70             75             80

cga cgc aaa att gat gcg gtg ggc atg tca ctc gtg ccg aag tta gac 288
Arg Arg Lys Ile Asp Ala Val Gly Met Ser Leu Val Pro Lys Leu Asp
      85             90             95

agg aca acg act tct ctt gca gcg aag gag gag aaa ttc agt tct atc 336
Arg Thr Thr Thr Ser Leu Ala Ala Lys Glu Glu Lys Phe Ser Ser Ile
      100             105             110

gac aag ata gtc tca aag cca acc cat tct ttt ggg gag agt tcc aaa 384
Asp Lys Ile Val Ser Lys Pro Thr His Ser Phe Gly Glu Ser Ser Lys
      115             120             125

tta cca gcg ggt ata atg aaa gcg aaa tca atg ttt ccg tca caa acc 432
Leu Pro Ala Gly Ile Met Lys Ala Lys Ser Met Phe Pro Ser Gln Thr
      130             135             140

ctt tcc gca ccg tgg aac gct cct gct cgt tgc gct cgg aaa gac agc 480
Leu Ser Ala Pro Trp Asn Ala Pro Ala Arg Cys Ala Arg Lys Asp Ser
      145             150             155             160

ttc ggg acg aag gcc tgg atc gaa aaa ctg caa aga gaa acc aca gac 528
Phe Gly Thr Lys Ala Trp Ile Glu Lys Leu Gln Arg Glu Thr Thr Asp

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<400> 90
Arg Gly Gly Ile Ser Val Pro Thr Leu Ser Ile Met Asn Gln Ser Thr
  1                   5                   10                   15
Ile Val Ala Thr Ser Val Val Ala Pro Gln Ser Ala Val Ser Leu Ser
      20                   25                   30
Arg Ala Pro Ser Arg Pro Gly Pro Ser Glu Ser Phe Gly Lys Gln Gln
      35                   40                   45
Glu Ser Arg Pro Gly Val Ser Gly Ala Gly Leu Ala Glu Ser Lys Arg
      50                   55                   60
Val Pro Ser Leu Thr Gln Pro Ser Leu Glu Arg Ser Val Thr Ile Ser
      65                   70                   75                   80

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Arg Arg Lys Ile Asp Ala Val Gly Met Ser Leu Val Pro Lys Leu Asp  
                   85                  90                  95

Arg Thr Thr Thr Ser Leu Ala Ala Lys Glu Glu Lys Phe Ser Ser Ile  
                   100                  105                  110

Asp Lys Ile Val Ser Lys Pro Thr His Ser Phe Gly Glu Ser Ser Lys  
                   115                  120                  125

Leu Pro Ala Gly Ile Met Lys Ala Lys Ser Met Phe Pro Ser Gln Thr  
                   130                  135                  140

Leu Ser Ala Pro Trp Asn Ala Pro Ala Arg Cys Ala Arg Lys Asp Ser  
                   145                  150                  155                  160

Phe Gly Thr Lys Ala Trp Ile Glu Lys Leu Gln Arg Glu Thr Thr Asp  
                   165                  170                  175

Thr Ser Gln Pro Pro Leu Glu Arg Gln Lys Ser Gln Arg Leu Ala Gln  
                   180                  185                  190

Thr Glu Pro Val Gln Lys Leu Lys Thr Ser Trp Leu Glu Pro Pro Gln  
                   195                  200                  205

Glu Val Glu Ser Gly His Gly Val Ala Glu Gly Asp Asp Leu Ser Val  
                   210                  215                  220

Ala Ala Ala Glu Tyr His Val Pro Glu Thr Glu Asp Gly Lys Pro Ser  
                   225                  230                  235                  240

Phe Lys Pro Ser Asp Pro Arg Val Trp Asn Arg Glu Trp Ile His Arg  
                   245                  250                  255

Arg Ile His Asn Pro Val Leu Ser Arg Ser Asn Arg  
                   260                  265

<210> 91

<211> 867

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(867)

<400> 91

cgg gat cca gct ggc aag gca gta aag aag gca gcc aca ggg ata cca 48

Arg	Asp	Pro	Ala	Gly	Lys	Ala	Val	Lys	Lys	Ala	Ala	Thr	Gly	Ile	Pro	
1				5				10					15			
aag	cct	gca	gct	cca	ggt	ggc	aag	gca	gtc	aag	gtg	act	cct	gtc	gcg	96
Lys	Pro	Ala	Ala	Pro	Gly	Gly	Lys	Ala	Val	Lys	Val	Thr	Pro	Val	Ala	
			20					25					30			
cga	aaa	cct	gtt	gca	cca	aag	gca	gca	gct	cca	gac	ggc	aag	gcg	gtc	144
Arg	Lys	Pro	Val	Ala	Pro	Lys	Ala	Ala	Ala	Pro	Asp	Gly	Lys	Ala	Val	
		35					40					45				
aag	aag	gca	acc	gta	gtc	gtg	cca	aag	cct	gca	gct	ccc	agt	ggc	aag	192
Lys	Lys	Ala	Thr	Val	Val	Val	Pro	Lys	Pro	Ala	Ala	Pro	Ser	Gly	Lys	
	50						55					60				
gca	gtg	aag	aag	ccg	gtt	gtc	agc	gtg	cca	aag	cct	gca	aca	ctc	ggt	240
Ala	Val	Lys	Lys	Pro	Val	Val	Ser	Val	Pro	Lys	Pro	Ala	Thr	Leu	Gly	
65					70				75						80	
ggc	aag	gca	gtg	aag	aag	cca	gct	gcc	ggc	gtg	cca	aag	ccc	gca	gct	288
Gly	Lys	Ala	Val	Lys	Lys	Pro	Ala	Ala	Gly	Val	Pro	Lys	Pro	Ala	Ala	
				85					90					95		
ccc	gat	ggc	aag	gcg	gtg	aga	aag	cca	gtt	gtc	ggc	gtg	cca	aag	ccc	336
Pro	Asp	Gly	Lys	Ala	Val	Arg	Lys	Pro	Val	Val	Gly	Val	Pro	Lys	Pro	
			100					105					110			
gca	gct	ccc	gat	ggt	aag	gcg	gcg	aaa	aag	cca	gcg	tcc	ggc	gtg	cca	384
Ala	Ala	Pro	Asp	Gly	Lys	Ala	Ala	Lys	Lys	Pro	Ala	Ser	Gly	Val	Pro	
		115					120					125				
aag	cct	gcg	gat	cca	gct	ggc	aag	gca	gta	aag	aag	gca	gcc	aca	ggg	432
Lys	Pro	Ala	Asp	Pro	Ala	Gly	Lys	Ala	Val	Lys	Lys	Ala	Ala	Thr	Gly	
	130					135					140					
ata	cca	aag	cct	gca	gct	cca	ggt	ggc	aag	gca	atc	aag	gtg	act	cct	480
Ile	Pro	Lys	Pro	Ala	Ala	Pro	Gly	Gly	Lys	Ala	Ile	Lys	Val	Thr	Pro	
145					150				155						160	
gtc	gcg	cga	aaa	cct	gtt	gca	cca	aag	gca	gca	gct	cca	gac	ggc	aag	528
Val	Ala	Arg	Lys	Pro	Val	Ala	Pro	Lys	Ala	Ala	Ala	Pro	Asp	Gly	Lys	
				165				170					175			
gca	gtc	aag	aag	gca	acc	gta	gtc	gtg	cca	aag	cct	gca	gct	ccc	agt	576
Ala	Val	Lys	Lys	Ala	Thr	Val	Val	Val	Pro	Lys	Pro	Ala	Ala	Pro	Ser	
			180					185				190				
ggc	aag	gca	gtg	aag	aag	cca	gtt	gtc	agc	gtg	cca	aag	cct	gca	acg	624

Gly Lys Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr  
 195 200 205

ctc gat ggc aag gcg gtg aga aag cca gtt gtc ggc gtg cca aag ccc 672  
 Leu Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro  
 210 215 220

gca gct ccc gat ggt aag gcg gtg aaa aag cca gtt gtc ggc gtg cca 720  
 Ala Ala Pro Asp Gly Lys Ala Val Lys Lys Pro Val Val Gly Val Pro  
 225 230 235 240

aag cct gca gct cca gat gac acg gga atc aac aag gcg acc ctt gtc 768  
 Lys Pro Ala Ala Pro Asp Asp Thr Gly Ile Asn Lys Ala Thr Leu Val  
 245 250 255

acg cgg aaa cct gag gct cca gac gtg aag gta gtc aag aag gca acc 816  
 Thr Arg Lys Pro Glu Ala Pro Asp Val Lys Val Val Lys Lys Ala Thr  
 260 265 270

gta gtt gtg cca aaa cct gaa gcg cca gat ata aag gta atg acg gat 864  
 Val Val Val Pro Lys Pro Glu Ala Pro Asp Ile Lys Val Met Thr Asp  
 275 280 285

ccg 867  
 Pro

&lt;210&gt; 92

&lt;211&gt; 289

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 92

Arg Asp Pro Ala Gly Lys Ala Val Lys Lys Ala Ala Thr Gly Ile Pro  
 1 5 10 15

Lys Pro Ala Ala Pro Gly Gly Lys Ala Val Lys Val Thr Pro Val Ala  
 20 25 30

Arg Lys Pro Val Ala Pro Lys Ala Ala Ala Pro Asp Gly Lys Ala Val  
 35 40 45

Lys Lys Ala Thr Val Val Val Pro Lys Pro Ala Ala Pro Ser Gly Lys  
 50 55 60

Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr Leu Gly  
 65 70 75 80

Gly Lys Ala Val Lys Lys Pro Ala Ala Gly Val Pro Lys Pro Ala Ala  
                             85                            90                            95  
  
 Pro Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro  
                             100                            105                            110  
  
 Ala Ala Pro Asp Gly Lys Ala Ala Lys Lys Pro Ala Ser Gly Val Pro  
                             115                            120                            125  
  
 Lys Pro Ala Asp Pro Ala Gly Lys Ala Val Lys Lys Ala Ala Thr Gly  
                             130                            135                            140  
  
 Ile Pro Lys Pro Ala Ala Pro Gly Gly Lys Ala Ile Lys Val Thr Pro  
                             145                            150                            155                            160  
  
 Val Ala Arg Lys Pro Val Ala Pro Lys Ala Ala Ala Pro Asp Gly Lys  
                             165                            170                            175  
  
 Ala Val Lys Lys Ala Thr Val Val Val Pro Lys Pro Ala Ala Pro Ser  
                             180                            185                            190  
  
 Gly Lys Ala Val Lys Lys Pro Val Val Ser Val Pro Lys Pro Ala Thr  
                             195                            200                            205  
  
 Leu Asp Gly Lys Ala Val Arg Lys Pro Val Val Gly Val Pro Lys Pro  
                             210                            215                            220  
  
 Ala Ala Pro Asp Gly Lys Ala Val Lys Lys Pro Val Val Gly Val Pro  
                             225                            230                            235                            240  
  
 Lys Pro Ala Ala Pro Asp Asp Thr Gly Ile Asn Lys Ala Thr Leu Val  
                             245                            250                            255  
  
 Thr Arg Lys Pro Glu Ala Pro Asp Val Lys Val Val Lys Lys Ala Thr  
                             260                            265                            270  
  
 Val Val Val Pro Lys Pro Glu Ala Pro Asp Ile Lys Val Met Thr Asp  
                             275                            280                            285  
  
 Pro

&lt;210&gt; 93

&lt;211&gt; 1434

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (i) .. (492)

&lt;400&gt; 93

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cgg ctt gtg ttg ccc gga gaa ggg gag aga cat gtt ttg cca aag gac 48
Arg Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp
  1             5             10             15

gag acg aaa cct gca ttg acg gat gaa aag aga acg aaa ccc ggt gaa 96
Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly Gly
             20             25             30

cca agg aag gag atg gag agg ccc gct gct ggt tct atg gaa aag gaa 144
Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp
             35             40             45

aag ctt gtt ttg ccc gga gaa ggg gag aga cat gtt ttg cca aag gaa 192
Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp
             50             55             60

gag acg aaa cct gca ttg acg gag gaa aag aga acg aaa ccc ggt gaa 240
Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro Gly Gly
             65             70             75

cca cga acg gag atg gag agg ccc gct gct ggt tct atg gaa aag gaa 288
Pro Arg Thr Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp
             85             90             95

aag cct ggt ttg ccc gga gaa ggg gag aga cat gtt ttg cca aag gaa 336
Lys Pro Gly Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp
             100            105            110

gag acg aaa cct gca ttg acg gag gaa aag aga acg aac ctg gaa gaa 384
Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu Ala Asp
             115            120            125

caa gaa agg aga tgg aga gcc cgc tgc tgg ttc ttg gaa aag gaa gaa 432
Gln Glu Arg Arg Trp Arg Ala Arg Cys Trp Phe Leu Glu Lys Glu Asp
             130            135            140

ctg ttt ggc ccg gag aag ggg aga gac acg ctt cgc caa aag gaa gaa 480
Leu Phe Gly Pro Glu Lys Gly Arg Asp Thr Leu Arg Gln Arg Thr Arg
             145            150            155

cga aag ccg cat tgacgcaaaa ggaggtgacg aatcccgttg aaccaagaaa 532
Arg Lys Pro His

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ggcgatggag aggcccgctg ctggttctat ggaaaaggaa aacctgtttc cccggagaag 592  
 gggagggaca tgttttgcca aagcacagac gaaacctgca ttgacagatg aaaagagaac 652  
 gaaacccggt ggaccaccaa cggagatgga gaggcccgct gctggtttta tggaaaagga 712  
 gaagcctggt ttgcccgag aaggggaggg acatgtttcc ccaaaggacg agatgaaacc 772  
 tgcattgacg gatgaaaaga gaacgaaacc cggtggaaca agaaaggaga tggagaggcc 832  
 cgctgctggt tttatggaaa aggagaagcc tgttttgccc ggagaagggg agagacatgt 892  
 tttgccaaaag gacgagcaga aagccgcatt gacgcagaag gaggtgacga atcccgttga 952  
 accaagaaag gagatggaga ggcccgctgt gcccatagaa ggggagaagg gtgttggtgc 1012  
 aagtgaagag gagaagcctg tttcgccaaa ggaagcgacg agacggattt tgccaaagga 1072  
 agggaaaagag atttggtact cggaaggagg aggtgaagcc gattgtgcga agggcaaaga 1132  
 gggggagacg gattgcacaa aaggggaagg agaaacaaat tgcacgaag gaggggaaga 1192  
 aaccgcgtgt accaaaggaa ggtgaggaaa gaccgcgtga accaacggaa ggcgaggaaa 1252  
 ggcccgttgg gccaaaggaa ggcgaggaaa gaccggtgt gccggacgta gacaaggaga 1312  
 aacctgttgt gcctgaagga gacaaggaga aacctgttgt gccggaagga gacaaggatc 1372  
 accctgcttc tgccagagca ggatgaggag aaacacgcta catgggagaa agaaatgatc 1432  
 cg 1434

&lt;210&gt; 94

&lt;211&gt; 164

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 94

Arg Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp  
 1 5 10 15

Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly Gly  
 20 25 30

Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp  
 35 40 45

Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp  
 50 55 60

Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro Gly Gly  
 65 70 75 80

Pro Arg Thr Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys Asp  
 85 90 95

Lys Pro Gly Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys Asp  
 100 105 110

Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Asn Leu Ala Asp  
 115 120 125

Gln Glu Arg Arg Trp Arg Ala Arg Cys Trp Phe Leu Glu Lys Glu Asn  
 130 135 140

Leu Phe Gly Pro Glu Lys Gly Arg Asp Thr Leu Arg Gln Arg Thr Arg  
 145 150 155 160

Arg Lys Pro His

<210> 95

<211> 680

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(678)

<400> 95

cga cca aga gca ggg agg gag cag cct gct gtt ccg cgg cag gaa gaa 48  
 Arg Pro Arg Ala Gly Arg Glu Gln Pro Ala Val Pro Arg Gln Glu Glu  
 1 5 10 15

cag aaa ctt gtt ttg caa aag aca gag agg aaa cca gtt ttg cca gag 96  
 Gln Lys Leu Val Leu Gln Lys Thr Glu Arg Lys Pro Val Leu Pro Glu  
 20 25 30

gaa gac cag aaa ccg gtt tta cca gaa aca ggg gcg aaa cat gtt tta 144  
 Glu Asp Gln Lys Pro Val Leu Pro Glu Thr Gly Ala Lys His Val Leu  
 35 40 45

ccg gaa ata gcg acc gaa tcc act ttg acg cag aaa gag ctg aca aaa 192

Pro	Glu	Ile	Ala	Thr	Glu	Ser	Thr	Leu	Thr	Gln	Lys	Glu	Leu	Thr	Lys	
	50					55					60					
ccc	gtt	gaa	aca	aga	cag	gac	atg	agg	ggg	acc	gct	ggt	tct	atg	gac	240
Pro	Val	Glu	Thr	Arg	Gln	Asp	Met	Arg	Gly	Thr	Ala	Gly	Ser	Met	Asp	
	65				70				75						80	
gag	aag	aag	cct	gtt	ttg	ccc	gga	gaa	tgg	gag	aga	cat	gtc	ttg	cca	288
Glu	Lys	Lys	Pro	Val	Leu	Pro	Gly	Glu	Trp	Glu	Arg	His	Val	Leu	Pro	
				85				90						95		
aaa	gac	gag	acg	aaa	cct	gca	ttg	acg	gag	gaa	aag	aga	acc	aaa	ccc	336
Lys	Asp	Glu	Thr	Lys	Pro	Ala	Leu	Thr	Glu	Glu	Lys	Arg	Thr	Lys	Pro	
			100					105					110			
gtt	gaa	cca	aga	aag	gag	atg	gag	agg	ccc	gct	cgc	ccc	atg	gaa	gag	384
Val	Glu	Pro	Arg	Lys	Glu	Met	Glu	Arg	Pro	Ala	Arg	Pro	Met	Glu	Glu	
		115					120					125				
gag	aag	cct	gtt	tta	ccc	gga	gaa	ggg	gag	aga	cat	gtt	ttg	cca	aag	432
Glu	Lys	Pro	Val	Leu	Pro	Gly	Glu	Gly	Glu	Arg	His	Val	Leu	Pro	Lys	
		130				135					140					
gac	ggg	atg	aaa	cct	gca	ttg	acg	gat	gaa	aag	aga	acg	aaa	ccc	ggt	480
Asp	Gly	Met	Lys	Pro	Ala	Leu	Thr	Asp	Glu	Lys	Arg	Thr	Lys	Pro	Gly	
	145				150					155				160		
gga	cca	agg	aag	gag	atg	gag	agg	ccc	gct	gct	ggt	tct	atg	gaa	aag	528
Gly	Pro	Arg	Lys	Glu	Met	Glu	Arg	Pro	Ala	Ala	Gly	Ser	Met	Glu	Lys	
				165				170						175		
gac	aag	ctt	gtg	ttg	ccc	gga	gaa	ggg	gag	aga	cat	gtt	ttg	cct	aag	576
Asp	Lys	Leu	Val	Leu	Pro	Gly	Glu	Gly	Glu	Arg	His	Val	Leu	Pro	Lys	
			180					185					190			
gac	gag	acg	aaa	cct	gca	ttg	acg	gat	gaa	aag	aga	acg	aaa	ccc	ggt	624
Asp	Glu	Thr	Lys	Pro	Ala	Leu	Thr	Asp	Glu	Lys	Arg	Thr	Lys	Pro	Gly	
		195					200					205				
gga	cca	aga	aag	gag	atg	gag	agg	ccc	gct	gct	ggt	tct	atg	gaa	aag	672
Gly	Pro	Arg	Lys	Ala	Met	Glu	Arg	Pro	Ala	Ala	Gly	Ser	Met	Glu	Lys	
	210						215				220					
gac	aag	cg														680
Asp	Lys															
	225															



<210> 96  
 <211> 226  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 96

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Arg Pro Arg Ala Gly Arg Glu Gln Pro Ala Val Pro Arg Gln Glu Glu
 1             5             10             15

Gln Lys Leu Val Leu Gln Lys Thr Glu Arg Lys Pro Val Leu Pro Glu
      20             25             30

Glu Asp Gln Lys Pro Val Leu Pro Glu Thr Gly Ala Lys His Val Leu
      35             40             45

Pro Glu Ile Ala Thr Glu Ser Thr Leu Thr Gln Lys Glu Leu Thr Lys
      50             55             60

Pro Val Glu Thr Arg Gln Asp Met Arg Gly Thr Ala Gly Ser Met Asp
      65             70             75             80

Glu Lys Lys Pro Val Leu Pro Gly Glu Trp Glu Arg His Val Leu Pro
      85             90             95

Lys Asp Glu Thr Lys Pro Ala Leu Thr Glu Glu Lys Arg Thr Lys Pro
      100            105            110

Val Glu Pro Arg Lys Glu Met Glu Arg Pro Ala Arg Pro Met Glu Glu
      115            120            125

Glu Lys Pro Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys
      130            135            140

Asp Gly Met Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly
      145            150            155            160

Gly Pro Arg Lys Glu Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys
      165            170            175

Asp Lys Leu Val Leu Pro Gly Glu Gly Glu Arg His Val Leu Pro Lys
      180            185            190

Asp Glu Thr Lys Pro Ala Leu Thr Asp Glu Lys Arg Thr Lys Pro Gly
      195            200            205

Gly Pro Arg Lys Ala Met Glu Arg Pro Ala Ala Gly Ser Met Glu Lys
      210            215            220

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Asp Lys  
225

<210> 97  
<211> 296  
<212> DNA  
<213> *Toxoplasma gondii*

<220>  
<221> CDS  
<222> (1)..(294)

<400> 97  
ccg gtc gac gtg gac gac ccc cgt ggc tgt tcg cag caa agc gga gac 48  
Pro Val Asp Val Asp Asp Pro Arg Gly Cys Ser Gln Gln Ser Gly Asp  
1 5 10 15  
acc aga gac agc agc agt ccc gcc aca cct ggt ggt cgg ccg gct ggt 96  
Thr Arg Asp Ser Ser Ser Pro Ala Thr Pro Gly Gly Arg Pro Ala Gly  
20 25 30  
ggg gca gga ggt gca gcg aca agc ccg aag gga cag gcc ttt gcc ccg 144  
Gly Ala Gly Gly Ala Ala Thr Ser Pro Lys Gly Gln Ala Phe Ala Pro  
35 40 45  
cgg ggc ggt gaa ggg gag ata aag ccc cag gag aca gga aac agt gga 192  
Arg Gly Gly Glu Gly Glu Ile Lys Pro Gln Glu Thr Gly Asn Ser Gly  
50 55 60  
gac agc aag gcg gag gga aag gaa gca agt gga gac gcg aac act tcg 240  
Asp Ser Lys Ala Glu Gly Lys Glu Ala Ser Gly Asp Ala Asn Thr Ser  
65 70 75 80  
gaa gga aag cga ttg tcg ggc gaa gtg gac aag aca gcc gag gtg gag 288  
Glu Gly Lys Arg Leu Ser Gly Glu Val Asp Lys Thr Ala Glu Val Glu  
85 90 95  
aca gcc gg 296  
Thr Ala

<210> 98  
<211> 98  
<212> PRT  
<213> *Toxoplasma gondii*

<400> 98

Pro Val Asp Val Asp Asp Pro Arg Gly Cys Ser Gln Gln Ser Gly Asp  
 1 5 10 15

Thr Arg Asp Ser Ser Ser Pro Ala Thr Pro Gly Gly Arg Pro Ala Gly  
 20 25 30

Gly Ala Gly Gly Ala Ala Thr Ser Pro Lys Gly Gln Ala Phe Ala Pro  
 35 40 45

Arg Gly Gly Glu Gly Glu Ile Lys Pro Gln Glu Thr Gly Asn Ser Gly  
 50 55 60

Asp Ser Lys Ala Glu Gly Lys Glu Ala Ser Gly Asp Ala Asn Thr Ser  
 65 70 75 80

Glu Gly Lys Arg Leu Ser Gly Glu Val Asp Lys Thr Ala Glu Val Glu  
 85 90 95

Thr Ala

<210> 99

<211> 723

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(159)

<400> 99

cga tcc tcc cga ggg acc gca gga agg ctc gcg tcc gaa gaa gac gac 48  
 Arg Ser Ser Arg Gly Thr Ala Gly Arg Leu Ala Ser Glu Glu Asp Asp  
 1 5 10 15

gga gac aac gaa gaa gag gaa cga gaa gaa gaa agg gag aga cgc gaa 96  
 Gly Asp Asn Glu Glu Glu Glu Arg Glu Glu Glu Arg Glu Arg Arg Glu  
 20 25 30

aga gaa gac ggg gaa gac gca ggc tct agg cgt cga gag aag gac ttc 144  
 Arg Glu Asp Gly Glu Asp Ala Gly Ser Arg Arg Arg Glu Lys Asp Phe  
 35 40 45

ttc cca gac acg act tgaatgcgta aaggcgtatt ttgtttccg atgaaaactc 199  
 Phe Pro Asp Thr Thr  
 50

gccaggggag gcgacttctc gcctctgagg aatccgacag tgacgagagg aagaggggaag 259  
 gagacgcaga gaaggacgcg tcaggaggat ccggaattcc ggatcgggag atggccccgg 319  
 agcgcgtagg ggcggtacac tgaagaacca acggaagaac actggggggtc gaaaatgtgt 379  
 ttccttttcg atgtgggtctt cccagctttc ctgcagacat gtgtacagaa cagctgagaa 439  
 aaaacgacga aagctccaat tgtctcttcg ttctcgagca gagaaaaccc cccgaggcct 499  
 tcgcttggtc agggcgaaac ctcaagggtg catgcagagt cggccgtgcc cagagtagcc 559  
 tagtcatgca gcccatcagt agcttaattt gacgcaatgg ctatttttac attgtgaaga 619  
 ggggtttcca atcaacaaac gccagagaag cctgtgttct ggaaaacctg aacgacggcc 679  
 gtcgttcccc tgtctgcttt accccctgac agtgcgtggt gagg 723

&lt;210&gt; 100

&lt;211&gt; 53

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 100

Arg Ser Ser Arg Gly Thr Ala Gly Arg Leu Ala Ser Glu Glu Asp Asp  
 1 5 10 15

Gly Asp Asn Glu Glu Glu Glu Arg Glu Glu Glu Arg Glu Arg Arg Glu  
 20 25 30

Arg Glu Asp Gly Glu Asp Ala Gly Ser Arg Arg Arg Glu Lys Asp Phe  
 35 40 45

Phe Pro Asp Thr Thr  
 50

&lt;210&gt; 101

&lt;211&gt; 270

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(270)

&lt;400&gt; 101

cgg aag ccg att gtg cga agg gca aag agg ggg aga cgg att gca caa 48  
 Arg Lys Pro Ile Val Arg Arg Ala Lys Arg Gly Arg Arg Ile Ala Gln  
 1 5 10 15

aag ggg aag gag aaa caa att gca tcg aag gag ggg aag aaa ccc gct 96  
 Lys Gly Lys Glu Lys Gln Ile Ala Ser Lys Glu Gly Lys Lys Pro Ala  
 20 25 30

gta cca aag gaa ggt gag gaa aga ccc gct gaa cca acg gaa ggc gag 144  
 Val Pro Lys Glu Gly Glu Glu Arg Pro Ala Glu Pro Thr Glu Gly Glu  
 35 40 45

gaa agg ccc gtt ggg cca aag gaa ggc gag gaa aga ccc gtt gtg ccg 192  
 Glu Arg Pro Val Gly Pro Lys Glu Gly Glu Glu Arg Pro Val Val Pro  
 50 55 60

gac gta gac aag gag aaa cct gtt gtg cct gaa gga gac aag gag aaa 240  
 Asp Val Asp Lys Glu Lys Pro Val Val Pro Glu Gly Asp Lys Glu Lys  
 65 70 75 80

cct gtt gtg ccg gaa gga gac aag gat ccg 270  
 Pro Val Val Pro Glu Gly Asp Lys Asp Pro  
 85 90

<210> 102

<211> 90

<212> PRT

<213> Toxoplasma gondii

<400> 102

Arg Lys Pro Ile Val Arg Arg Ala Lys Arg Gly Arg Arg Ile Ala Gln  
 1 5 10 15

Lys Gly Lys Glu Lys Gln Ile Ala Ser Lys Glu Gly Lys Lys Pro Ala  
 20 25 30

Val Pro Lys Glu Gly Glu Glu Arg Pro Ala Glu Pro Thr Glu Gly Glu  
 35 40 45

Glu Arg Pro Val Gly Pro Lys Glu Gly Glu Glu Arg Pro Val Val Pro  
 50 55 60

Asp Val Asp Lys Glu Lys Pro Val Val Pro Glu Gly Asp Lys Glu Lys  
 65 70 75 80

Pro Val Val Pro Glu Gly Asp Lys Asp Pro  
 85 90

<210> 103  
 <211> 503  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(186)

<400> 103  
 cgg cat ctc tgg tgc gtg cgc gag aga tcc ccg caa cga gaa aga tgg 48  
 Arg His Leu Trp Cys Val Arg Glu Arg Ser Pro Gln Arg Glu Arg Trp  
 1 5 10 15  
 agc ttc gtc tcg ttc tcg ctt ttc ttc tct ttc cag ttc ttt ttc agc 96  
 Ser Phe Val Ser Phe Ser Leu Phe Phe Ser Phe Gln Phe Phe Phe Ser  
 20 25 30  
 aag caa gtc tcg cgc ctc cct cgt ccg agc agc gtc act gca ctg tgg 144  
 Lys Gln Val Ser Arg Leu Pro Arg Pro Ser Ser Val Thr Ala Leu Trp  
 35 40 45  
 gcc atc agc aga aag aag gcg aag aaa aga gac gac ggc aga 186  
 Ala Ile Ser Arg Lys Lys Ala Lys Lys Arg Asp Asp Gly Arg  
 50 55 60  
 taatggcgcg aaaatctatc ccaaaaacac atatatgcct tatggcagtg agcgaagaga 246  
 gggaactgcc aacgccttgg cggaagcccg ttctccaaac gaggttgagg taccaaacct 306  
 gcatgcggag agaccaaggc aggttttgtc ttccgtcgct tccgtggatg cttttcgcac 366  
 gtatgcaaaa gagagaacgg gaccaagtgc aagaagttat agagcagtcg cgacgacaga 426  
 gacgcancta gaggccgagc aagaatcggt tttttcttct cgtaagggaa acgcagtgca 486  
 tanaagcaaa agaccgg 503

<210> 104  
 <211> 62  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 104  
 Arg His Leu Trp Cys Val Arg Glu Arg Ser Pro Gln Arg Glu Arg Trp

<210> 106

&lt;211&gt; 73

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 106

Arg Arg Asp Leu Arg Thr Ser Val Trp Asp Ala Arg Val Tyr Val His  
 1 5 10 15

Leu Ala Gly Gly Gln Arg Arg Cys Asn Glu Ser Arg Gly Met Glu Glu  
 20 25 30

Ala Arg Lys Arg Arg Cys Leu Ala Met Arg Cys Gln Trp Thr Xaa Ser  
 35 40 45

Ala Leu Asp Trp Arg Glu Ser Trp Lys Asn Ala Glu Thr Ala Ser His  
 50 55 60

Val Thr Phe Pro Thr Lys Arg Pro Pro  
 65 70

&lt;210&gt; 107

&lt;211&gt; 390

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;223&gt; N = unknown at 104

&lt;220&gt;

&lt;223&gt; Xaa = unknown at 35

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(201)

&lt;400&gt; 107

cgg cga atc ccc cag gaa ttg ttg aaa cag agt ctc aga ttc tac gga 48  
 Arg Arg Ile Pro Gln Glu Leu Leu Lys Gln Ser Leu Arg Phe Tyr Gly  
 1 5 10 15

ctc cga ggg cct ctg ctt gcc cgc cct gtg cac agg cgt cag cac gtg 96  
 Leu Arg Gly Pro Leu Leu Ala Arg Pro Val His Arg Arg Gln His Val  
 20 25 30

gtt ctc ana gaa aaa gtt ggt aag tgg aag tgg tgg agc caa gaa aaa 144  
 Val Leu Xaa Glu Lys Val Gly Lys Trp Lys Trp Trp Ser Gln Glu Lys  
 35 40 45



ctc aac tct tct tgt ttt ccg gag aat ttt cct ggt gtt caa ttc cac 192  
 Leu Asn Ser Ser Cys Phe Pro Glu Asn Phe Pro Gly Val Gln Phe His  
           50                          55                          60

ggt tct gga tagtctttgt tgtattaaaa cacatctaga aggactgaga 241  
 Gly Ser Gly  
           65

cgttgctcggg agttgaatta cagacacttc gttttccagc gtcagcttgc atgcccgtcc 301

cctgtttctg gaacacaagc ttgagaagg aaacgagaca gagaacgacg aaggaagtga 361

agcaaatcct ctgacggatt tccattcgg 390

<210> 108

<211> 67

<212> PRT

<213> Toxoplasma gondii

<400> 108

Arg Arg Ile Pro Gln Glu Leu Leu Lys Gln Ser Leu Arg Phe Tyr Gly  
       1                          5                          10                          15

Leu Arg Gly Pro Leu Leu Ala Arg Pro Val His Arg Arg Gln His Val  
                           20                          25                          30

Val Leu Xaa Glu Lys Val Gly Lys Trp Lys Trp Trp Ser Gln Glu Lys  
                           35                          40                          45

Leu Asn Ser Ser Cys Phe Pro Glu Asn Phe Pro Gly Val Gln Phe His  
           50                          55                          60

Gly Ser Gly  
       65

<210> 109

<211> 699

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(699)

<400> 109

ccg tgc gtc tgt gag gaa aag tgc aag aca ggg ccg aac tgt gac cag	48
Pro Cys Val Cys Glu Glu Lys Cys Lys Thr Gly Pro Asn Cys Asp Gln	
1 5 10 15	
cat aaa ccg gag tgc tgt ggg tcg aac gac gac tgc cat cag cct cag	96
His Lys Pro Glu Cys Cys Gly Ser Asn Asp Asp Cys His Gln Pro Gln	
20 25 30	
ggg tac tgc aag atg gac atg tcc aca tgc atc tgc cgt cca ggc ttc	144
Gly Tyr Cys Lys Met Asp Met Ser Thr Cys Ile Cys Arg Pro Gly Phe	
35 40 45	
acg ggc gag aac tgc gga aca cgg gaa gat ctg tgc gca ggt gtg acg	192
Thr Gly Glu Asn Cys Gly Thr Arg Glu Asp Leu Cys Ala Gly Val Thr	
50 55 60	
tgc aag aac ggc ggg aca tgc gac tcc gtc act ggc ctg tgc cag tgc	240
Cys Lys Asn Gly Gly Thr Cys Asp Ser Val Thr Gly Leu Cys Gln Cys	
65 70 75 80	
gat gcc tgc cac ggc ggg aag acc tgc gag att acg aag gaa cac tgc	288
Asp Ala Cys His Gly Gly Lys Thr Cys Glu Ile Thr Lys Glu His Cys	
85 90 95	
tgc atc aat gac agt gac tgc aac ggc cac ggc acc tgc aac acg agc	336
Cys Ile Asn Asp Ser Asp Cys Asn Gly His Gly Thr Cys Asn Thr Ser	
100 105 110	
aac aat acc tgc aac tgc gag gca ggc ttc gct ggc acc aac tgc tcg	384
Asn Asn Thr Cys Asn Cys Glu Ala Gly Phe Ala Gly Thr Asn Cys Ser	
115 120 125	
agc agc gaa ggc aag tgc agc ggc aag acc tgc ttg agt gga cac tgc	432
Ser Ser Glu Gly Lys Cys Ser Gly Lys Thr Cys Leu Ser Gly His Cys	
130 135 140	
aat ccg gcg act ggc gca tgc gtc tgc gac ccg tgc cac acc ggc gag	480
Asn Pro Ala Thr Gly Ala Cys Val Cys Asp Pro Cys His Thr Gly Glu	
145 150 155 160	
aga tgc gaa acg ctc gtc aag gac tgc tgt gtt gtg aac gac acg tgc	528
Arg Cys Glu Thr Leu Val Lys Asp Cys Cys Val Val Asn Asp Thr Cys	
165 170 175	
aag ttc ccc aac ggc gtc tgc act gac agc aac agg tgt gag tgc cag	576
Lys Phe Pro Asn Gly Val Cys Thr Asp Ser Asn Arg Cys Glu Cys Gln	
180 185 190	

**PCT/US98/27137**

tgc att tgc ccc cca ggc ttt gga gac 699  
Cys Ile Cys Pro Pro Gly Phe Gly Asp  
225 230

<213> Toxoplasma gondii

Asn Pro Ala Thr Gly Ala Cys Val Cys Asp Pro Cys His Thr Gly Glu  
145 150 155 160

Arg Cys Glu Thr Leu Val Lys Asp Cys Cys Val Val Asn Asp Thr Cys  
165 170 175

Lys Phe Pro Asn Gly Val Cys Thr Asp Ser Asn Arg Cys Glu Cys Gln  
180 185 190

Ser Gly Trp Gly Gln Gly Asp Cys Ser Lys Pro Val Asp Lys Cys Glu  
195 200 205

Asp Val Ser Cys Asn Asn Gly Ser Ser Cys Asp Ala Asp Ser Gly Thr  
210 215 220

Cys Ile Cys Pro Pro Gly Phe Gly Asp  
225 230

<210> 111

<211> 419

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(417)

<400> 111

gag atg agc gcc cca gat agg caa aca gga aag ctt tcc gat tta cca 48  
Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro  
1 5 10 15

cca ttt gct gag ctg cca cag ctg gca gaa ata cca aag ctc tcc gaa 96  
Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Gln  
20 25 30

ctt ccg aaa atc gcg gac atg ccg aaa ttt tcg gat atg ccc aag ata 144  
Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met  
35 40 45

gcc gag atg ccc aag tta tca gat ata ccc aag atg gct gag atg tta 192  
Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro  
50 55 60

aag tta tca gat ata ccc aag atg gct gag atg ccc aag tta tca gat 240  
Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp  
65 70 75 80

ata ccc aag atg gct gag atg ccc aag ttt tca gat ata ccc aag atg 288

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met  
                             85                            90                            95

gct gag atg cca aag tta tca gat atg ccc aga atg gct gac att cca 336  
 Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro  
                             100                            105                            110

cag ttt cca gag atg cct agg atg gtt gac atg cct cag ttt cca gaa 384  
 Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu  
                             115                            120                            125

atc ccc agg atg gct gat atg ccg caa ttt ccg cg 419  
 Ile Pro Arg Met Ala Asp Met Pro Gln Phe Pro  
                             130                            135

&lt;210&gt; 112

&lt;211&gt; 139

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 112

Glu Met Ser Ala Pro Asp Arg Gln Thr Gly Lys Leu Ser Asp Leu Pro  
   1                            5                            10                            15

Pro Phe Ala Glu Leu Pro Gln Leu Ala Glu Ile Pro Lys Leu Ser Glu  
                             20                            25                            30

Leu Pro Lys Ile Ala Asp Met Pro Lys Phe Ser Asp Met Pro Lys Met  
                             35                            40                            45

Ala Glu Met Pro Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro  
                             50                            55                            60

Lys Leu Ser Asp Ile Pro Lys Met Ala Glu Met Pro Lys Leu Ser Asp  
   65                            70                            75                            80

Ile Pro Lys Met Ala Glu Met Pro Lys Phe Ser Asp Ile Pro Lys Met  
                             85                            90                            95

Ala Glu Met Pro Lys Leu Ser Asp Met Pro Arg Met Ala Asp Ile Pro  
                             100                            105                            110

Gln Phe Pro Glu Met Pro Arg Met Val Asp Met Pro Gln Phe Pro Glu  
                             115                            120                            125

Ile Pro Arg Met Ala Asp Met Pro Gln Phe Pro  
                             130                            135

<210> 113  
 <211> 303  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <221> CDS  
 <222> (1)..(303)

<400> 113  
 gac gaa gct ctt cct ctc ttt gga gca aac ggt gga acc tca gtt cgg 48  
 Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Gly Gly Thr Ser Val Arg  
     1                    5                    10                    15  
  
 ctc tcc ctc gac cgc agc gtc ctg ctt gtt ctc gaa ccc gca gag ccc 96  
 Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro  
                     20                    25                    30  
  
 ctg cta tcc tct tgg ccc cac ccg ggg aga aga gac act ttt ctt gaa 144  
 Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu  
             35                    40                    45  
  
 ggc gat ggc gcg ggc atc ccg tct cct tca tct cgg ccg agt cgc gcg 192  
 Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala  
     50                    55                    60  
  
 gcc gac cat tac acg aga ctc tcc acg att ccg tct ctt gcc agg gat 240  
 Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp  
     65                    70                    75                    80  
  
 gga gag gtc gac tcc gag ctg gcg ggg gga ccg cag gaa aga gaa agt 288  
 Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser  
                     85                    90                    95  
  
 gtc aga gtg gat ccg 303  
 Val Arg Val Asp Pro  
             100

<210> 114  
 <211> 101  
 <212> PRT  
 <213> *Toxoplasma gondii*

<400> 114  
 Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Gly Gly Thr Ser Val Arg

<400> 115																	
cgc	ggt	aac	gaa	aaa	aca	tgc	tca	gat	gcc	aag	cat	cca	gtg	tac	atc		48
Arg	Gly	Asn	Glu	Lys	Thr	Cys	Ser	Asp	Ala	Lys	His	Pro	Val	Tyr	Ile		
1				5					10					15			
aaa	ctt	ggc	aaa	ggg	gaa	cgc	gag	gcc	gta	ttc	aag	tgt	ggc	gac	ggc		96
Lys	Leu	Gly	Lys	Gly	Glu	Arg	Glu	Ala	Val	Phe	Lys	Cys	Gly	Asp	Gly		
			20					25					30				
ctc	act	act	ctt	gag	cca	tcg	cag	aac	aca	gat	aaa	cca	aaa	ttc	tgt		144
Leu	Thr	Thr	Leu	Glu	Pro	Ser	Gln	Asn	Thr	Asp	Lys	Pro	Lys	Phe	Cys		
			35				40					45					
gaa	tcg	ata	gac	tgc	aac	gat	act	gca	gaa	ctt	gaa	aca	acg	ttc	cca		192
Glu	Ser	Ile	Asp	Cys	Asn	Asp	Thr	Ala	Glu	Leu	Glu	Thr	Thr	Phe	Pro		
	50					55					60						
ggg	gcg	tac	tgg	gac	gag	aga	aac	aaa	aaa	gcg	aat	ata	tac	aga	ctg		240

Gly Ala Tyr Trp Asp Glu Arg Asn Lys Lys Ala Asn Ile Tyr Arg Leu  
 65 70 75 80  
 gtc att cct acc gtg agc aga aaa gac act cgg atg tat tat aaa tgt 288  
 Val Ile Pro Thr Val Ser Arg Lys Asp Thr Arg Met Tyr Tyr Lys Cys  
 85 90 95  
 aaa ggc act tcg gat tcc gcc gac cca tgc aca gta ctg ata aac gtg 336  
 Lys Gly Thr Ser Asp Ser Ala Asp Pro Cys Thr Val Leu Ile Asn Val  
 100 105 110  
 aaa tct aca gag act gat gat gat gag gaa gag gac gtg cag gag tgc 384  
 Lys Ser Thr Glu Thr Asp Asp Asp Glu Glu Glu Asp Val Gln Glu Cys  
 115 120 125  
 acg gtg ggc acc gag aag aaa gtc aca ctg tcc ccc acc gat acc gtg 432  
 Thr Val Gly Thr Glu Lys Lys Val Thr Leu Ser Pro Thr Asp Thr Val  
 130 135 140  
 aaa ttc aag tgc aat ctc gga aca gtt gtg cag cca tca ttc tcc aca 480  
 Lys Phe Lys Cys Asn Leu Gly Thr Val Val Gln Pro Ser Phe Ser Thr  
 145 150 155 160  
 gca act ccg aaa gtc ttt gac gac tcc gat ggc tcc tgc agt gca cag 528  
 Ala Thr Pro Lys Val Phe Asp Asp Ser Asp Gly Ser Cys Ser Ala Gln  
 165 170 175  
 gct agc ctg acg tct ctg gta gat gcc tcg ctc acg gaa gac agt tca 576  
 Ala Ser Leu Thr Ser Leu Val Asp Ala Ser Leu Thr Glu Asp Ser Ser  
 180 185 190  
 cat ggc aag tac aca atg tat acc atg aac ctg aac gca cgc cca gct 624  
 His Gly Lys Tyr Thr Met Tyr Thr Met Asn Leu Asn Ala Arg Pro Ala  
 195 200 205  
 gag aca aag aat ctc tgt ctc caa tgt tcc tct gga aag cag aac tgc 672  
 Glu Thr Lys Asn Leu Cys Leu Gln Cys Ser Ser Gly Lys Gln Asn Cys  
 210 215 220  
 aaa atg cgc atc cat gta ccc gcg 696  
 Lys Met Arg Ile His Val Pro Ala  
 225 230

&lt;210&gt; 116

&lt;211&gt; 232

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii



&lt;400&gt; 116

Arg Gly Asn Glu Lys Thr Cys Ser Asp Ala Lys His Pro Val Tyr Ile  
 1 5 10 15

Lys Leu Gly Lys Gly Glu Arg Glu Ala Val Phe Lys Cys Gly Asp Gly  
 20 25 30

Leu Thr Thr Leu Glu Pro Ser Gln Asn Thr Asp Lys Pro Lys Phe Cys  
 35 40 45

Glu Ser Ile Asp Cys Asn Asp Thr Ala Glu Leu Glu Thr Thr Phe Pro  
 50 55 60

Gly Ala Tyr Trp Asp Glu Arg Asn Lys Lys Ala Asn Ile Tyr Arg Leu  
 65 70 75 80

Val Ile Pro Thr Val Ser Arg Lys Asp Thr Arg Met Tyr Tyr Lys Cys  
 85 90 95

Lys Gly Thr Ser Asp Ser Ala Asp Pro Cys Thr Val Leu Ile Asn Val  
 100 105 110

Lys Ser Thr Glu Thr Asp Asp Asp Glu Glu Glu Asp Val Gln Glu Cys  
 115 120 125

Thr Val Gly Thr Glu Lys Lys Val Thr Leu Ser Pro Thr Asp Thr Val  
 130 135 140

Lys Phe Lys Cys Asn Leu Gly Thr Val Val Gln Pro Ser Phe Ser Thr  
 145 150 155 160

Ala Thr Pro Lys Val Phe Asp Asp Ser Asp Gly Ser Cys Ser Ala Gln  
 165 170 175

Ala Ser Leu Thr Ser Leu Val Asp Ala Ser Leu Thr Glu Asp Ser Ser  
 180 185 190

His Gly Lys Tyr Thr Met Tyr Thr Met Asn Leu Asn Ala Arg Pro Ala  
 195 200 205

Glu Thr Lys Asn Leu Cys Leu Gln Cys Ser Ser Gly Lys Gln Asn Cys  
 210 215 220

Lys Met Arg Ile His Val Pro Ala  
 225 230

<210> 117  
 <211> 173  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(171)

<400> 117  
 act tgt gcg ggg gac ccc tcg gcc ttt ccg acg aag ctg ccg tcg aca 48  
 Thr Cys Ala Gly Asp Pro Ser Ala Phe Pro Thr Lys Leu Pro Ser Thr  
 1 5 10 15  
 cca ccc gct gct gtg ccg tct gac ggg ttg ctc gct ttg ccc tca gaa 96  
 Pro Pro Ala Ala Val Pro Ser Asp Gly Leu Leu Ala Leu Pro Ser Glu  
 20 25 30  
 ctt gag gcg ccg gtg gag gac ggc gac cgc gag gct ttc gtt gga gtc 144  
 Leu Glu Ala Pro Val Glu Asp Gly Asp Arg Glu Ala Phe Val Gly Val  
 35 40 45  
 gac ggc gcg gtc agc ggc tgg gac gag cg 173  
 Asp Gly Ala Val Ser Gly Trp Asp Glu  
 50 55

<210> 118  
 <211> 57  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 118  
 Thr Cys Ala Gly Asp Pro Ser Ala Phe Pro Thr Lys Leu Pro Ser Thr  
 1 5 10 15  
 Pro Pro Ala Ala Val Pro Ser Asp Gly Leu Leu Ala Leu Pro Ser Glu  
 20 25 30  
 Leu Glu Ala Pro Val Glu Asp Gly Asp Arg Glu Ala Phe Val Gly Val  
 35 40 45  
 Asp Gly Ala Val Ser Gly Trp Asp Glu  
 50 55

<210> 119  
 <211> 369

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(369)

&lt;400&gt; 119

```

cgc tct gtg ttt cag gtc gcg agc gac gcg aga aac gcc cga cag gcg      48
Arg Ser Val Phe Gln Val Ala Ser Asp Ala Arg Asn Ala Arg Gln Ala
   1               5               10               15

acc tcg ggc gtg ccg cgg cag agg gga aag aag gcc gtc acg gcg cga      96
Thr Ser Gly Val Pro Arg Gln Arg Gly Lys Lys Ala Val Thr Ala Arg
           20               25               30

gtc tct ttc ggc gct cta gag gag aga gac agt tcg agt tcg gac gtt      144
Val Ser Phe Gly Ala Leu Glu Glu Arg Asp Ser Ser Ser Ser Asp Val
       35               40               45

ccc gag gaa agg gat aaa gac gcc gaa aac ggc tct gcg cct cgc atc      192
Pro Glu Glu Arg Asp Lys Asp Ala Glu Asn Gly Ser Ala Pro Arg Ile
       50               55               60

ttc gcg tct tct tcc ctg acg cgg ctt tcg cct cct tct ctc tct ccg      240
Phe Ala Ser Ser Ser Leu Thr Arg Leu Ser Pro Pro Ser Leu Ser Pro
       65               70               75               80

ctc tca agt tcg ggg cca tct tca ccg tct tct tcc gtt tcg cgg ttt      288
Leu Ser Ser Ser Gly Pro Ser Ser Pro Ser Ser Ser Val Ser Arg Phe
           85               90               95

acc gac tcc ctg ccg cag tcg acg gct tcg tct cgt ctc tcc tct gct      336
Thr Asp Ser Leu Pro Gln Ser Thr Ala Ser Ser Arg Leu Ser Ser Ala
           100               105               110

tat tcg ctt gag tcg cgt cgg cct ctg gag ccg                        369
Tyr Ser Leu Glu Ser Arg Arg Pro Leu Glu Pro
       115               120

```

&lt;210&gt; 120

&lt;211&gt; 123

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 120

Arg Ser Val Phe Gln Val Ala Ser Asp Ala Arg Asn Ala Arg Gln Ala

106

50

55

60

gctgaagaca tttgtagacg ctttctacaa acccagctgg caaaatctta cggaaggaca 253  
aatgcctctt tcaacactct tctttcatcg ctgcttggtta cactcctgag aggccccaag 313  
agccacggtg ccactttgct tccccagccg ctactgtgca aattctttat agaagagcac 373  
aaatgttccc cgaagaagca gcagcaccct ttgaggagcc tgaagagcga ccctacgaat 433  
cacagcgttc agaaatagcc tactgtagta ttaaggagac taccaaagtg aaaatcgtga 493  
tatgtctaca ggtggtatgc aagtgttggt tttccagata tacgctgcaa ctaaaacacc 553  
aaaatgatag aat 566

&lt;210&gt; 122

&lt;211&gt; 61

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 122

Arg Arg Trp Met Thr Gly Ala Asn Tyr Glu Gly His Gln Gly Gln Tyr  
1 5 10 15

Leu Asn Tyr Cys Thr Ile Ser His Phe Leu Cys Cys Pro Asn Gly Ile  
20 25 30

Cys Arg Phe Gln Trp Asp Asn Gln Pro Ser Leu Asp Arg Glu Asp Ser  
35 40 45

Ile Trp Cys Ser Glu Ser Ile Ser Arg Phe Arg Leu Ser  
50 55 60

&lt;210&gt; 123

&lt;211&gt; 616

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(615)

&lt;400&gt; 123

cac gag cgc cgt gtg gca gag caa aag gct cgt gaa gaa cgc gag aga 48  
His Glu Arg Arg Val Ala Glu Gln Lys Ala Arg Glu Glu Arg Glu Arg

1	5	10	15	
cag gca gca tct cag cga aac gga tcg aca gaa ccc gct gtt gct ccc				96
Gln Ala Ala Ser Gln Arg Asn Gly Ser Thr Glu Pro Ala Val Ala Pro				
	20	25	30	
tcc tct tgt tcc tcc agc aac tca cag aac cct ccg caa gat tcc tcg				144
Ser Ser Cys Ser Ser Ser Asn Ser Gln Asn Pro Pro Gln Asp Ser Ser				
	35	40	45	
cac gtc tgc tgt ccc tcc tcc tct gcc ttc tcc cag ccg cgc tct tct				192
His Val Cys Cys Pro Ser Ser Ser Ala Phe Ser Gln Pro Arg Ser Ser				
	50	55	60	
ctg tcc tca tcc tca ccc tct tcg tct gcc gcg tta cca tcg ggg tct				240
Leu Ser Ser Ser Ser Pro Ser Ser Ser Ala Ala Leu Pro Ser Gly Ser				
	65	70	75	80
tct ccc tcg gct gcg tct tcg tct cat gca ctt ggg gtg gtg gac tcg				288
Ser Pro Ser Ala Ala Ser Ser Ser His Ala Leu Gly Val Val Asp Ser				
	85	90	95	
gac cgg att tct gcg gag gag gcg gcg tcc ctc gag gag gcc cgg cgg				336
Asp Arg Ile Ser Ala Glu Glu Ala Ala Ser Leu Glu Glu Ala Arg Arg				
	100	105	110	
ctg cag aga cag ttc gag gcg gaa atg gtg ggc att cga ccg cca gac				384
Leu Gln Arg Gln Phe Glu Ala Glu Met Val Gly Ile Arg Pro Pro Asp				
	115	120	125	
gac acc tac gag gaa acg ctg att tct gag gac atc cat cct tcc cac				432
Asp Thr Tyr Glu Glu Thr Leu Ile Ser Glu Asp Ile His Pro Ser His				
	130	135	140	
cga gcc tgg tgg gaa aga cct agc gcc tcg ccg att cgt ctg tcg cgc				480
Arg Ala Trp Trp Glu Arg Pro Ser Ala Ser Pro Ile Arg Leu Ser Arg				
	145	150	155	160
gcg gcg tcg atg aga agt gac ggt cgc aga ggt caa cag ccc ccg agt				528
Ala Ala Ser Met Arg Ser Asp Gly Arg Arg Gly Gln Gln Pro Pro Ser				
	165	170	175	
cga cag tct cct cag gac ggg gag gaa gac gac gcc gct ctc gcc aga				576
Arg Gln Ser Pro Gln Asp Gly Glu Glu Asp Asp Ala Ala Leu Ala Arg				
	180	185	190	
cga ctt cag gaa gaa gaa tac agc cga cat cga gag gtc g				616
Arg Leu Gln Glu Glu Glu Tyr Ser Arg His Arg Glu Val				

195

200

205

&lt;210&gt; 124

&lt;211&gt; 205

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 124

His Glu Arg Arg Val Ala Glu Gln Lys Ala Arg Glu Glu Arg Glu Arg  
 1 5 10 15

Gln Ala Ala Ser Gln Arg Asn Gly Ser Thr Glu Pro Ala Val Ala Pro  
 20 25 30

Ser Ser Cys Ser Ser Ser Asn Ser Gln Asn Pro Pro Gln Asp Ser Ser  
 35 40 45

His Val Cys Cys Pro Ser Ser Ser Ala Phe Ser Gln Pro Arg Ser Ser  
 50 55 60

Leu Ser Ser Ser Ser Pro Ser Ser Ser Ala Ala Leu Pro Ser Gly Ser  
 65 70 75 80

Ser Pro Ser Ala Ala Ser Ser Ser His Ala Leu Gly Val Val Asp Ser  
 85 90 95

Asp Arg Ile Ser Ala Glu Glu Ala Ala Ser Leu Glu Glu Ala Arg Arg  
 100 105 110

Leu Gln Arg Gln Phe Glu Ala Glu Met Val Gly Ile Arg Pro Pro Asp  
 115 120 125

Asp Thr Tyr Glu Glu Thr Leu Ile Ser Glu Asp Ile His Pro Ser His  
 130 135 140

Arg Ala Trp Trp Glu Arg Pro Ser Ala Ser Pro Ile Arg Leu Ser Arg  
 145 150 155 160

Ala Ala Ser Met Arg Ser Asp Gly Arg Arg Gly Gln Gln Pro Ser Ser  
 165 170 175

Arg Gln Ser Pro Gln Asp Gly Glu Glu Asp Asp Ala Ala Leu Ala Arg  
 180 185 190

Arg Leu Gln Glu Glu Glu Tyr Ser Arg His Arg Glu Val  
 195 200 205

&lt;210&gt; 125

&lt;211&gt; 762

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(762)

&lt;400&gt; 125

```

cgg gat cag gct cct aag cca gtg ccc gag gca gcc gac gaa ttt gac      48
Arg Asp Gln Ala Pro Lys Pro Val Pro Glu Ala Ala Asp Glu Phe Asp
  1              5              10              15

cag gct cct atg cca ctg ccc gaa gca ccc gaa gac ttt gac cag gct      96
Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala
          20              25              30

cct gag cca ctg cgc gag gca gcc gaa gaa ttt gac cag gct cct atg      144
Pro Glu Pro Leu Arg Glu Ala Ala Glu Glu Phe Asp Gln Ala Pro Met
      35              40              45

cca gtg ccc gag gca ccc gaa gac ttt gac cag att cct aag cca gtg      192
Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ile Pro Lys Pro Val
      50              55              60

ccc gag gca ccc gaa gaa ttt gac cag gct cct atg cca gtg ccc gag      240
Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Val Pro Glu
      65              70              75              80

gca ccc gaa gac ttt gac cag att cct aag cca gtg ccc gag gca ccc      288
Ala Pro Glu Asp Phe Asp Gln Ile Pro Lys Pro Val Pro Glu Ala Pro
          85              90              95

gaa gaa ttt gac cag gct cct atg cca ctc ccc gaa gca ccc gaa gaa      336
Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Glu
      100              105              110

tcc gag cag gct cct gag cca ctg ccc gag gca ccc gaa gaa tcc gag      384
Ser Glu Gln Ala Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu
      115              120              125

cag gct cct gag cca ctg ccc gag gca ccc gaa gaa tcc gag cag gct      432
Gln Ala Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala
      130              135              140

cct gag cca ctg ccc gag gca ccc gaa gaa tcc gag cag gct cct gag      480

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Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu  
 145 150 155 160

cca ctg ccc gag gca ccc gaa gaa tcc gag cag gct cct gag cca ctg 528  
 Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu Pro Leu  
 165 170 175

ccc gag gca ccc gaa gaa ttt gac cag gct cct atg cca ctg ccc gcg 576  
 Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Pro Ala  
 180 185 190

gcc ccc gaa gac ttt gac cag cct gct atg cca ctg ccc ccg gcc ccc 624  
 Ala Pro Glu Asp Phe Asp Gln Pro Ala Met Pro Leu Pro Pro Ala Pro  
 195 200 205

gaa gac ttt gac cag gct ccc atg cca ctg ccg cag gca ccc gaa gaa 672  
 Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Gln Ala Pro Glu Glu  
 210 215 220

ctc gag cag gct ccc gct tcc acc ccg agg agg cgg agc agg agg tgc 720  
 Leu Glu Gln Ala Pro Ala Ser Thr Pro Arg Arg Arg Ser Arg Arg Cys  
 225 230 235 240

ctg aga gaa aaa ctg acg cag gaa gtg aac ctg aga agg atc 762  
 Leu Arg Glu Lys Leu Thr Gln Glu Val Asn Leu Arg Arg Ile  
 245 250

&lt;210&gt; 126

&lt;211&gt; 254

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 126

Arg Asp Gln Ala Pro Lys Pro Val Pro Glu Ala Ala Asp Glu Phe Asp  
 1 5 10 15

Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala  
 20 25 30

Pro Glu Pro Leu Arg Glu Ala Ala Glu Glu Phe Asp Gln Ala Pro Met  
 35 40 45

Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ile Pro Lys Pro Val  
 50 55 60

Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Val Pro Glu  
 65 70 75 80

Ala Pro Glu Asp Phe Asp Gln Ile Pro Lys Pro Val Pro Glu Ala Pro  
85 90 95

Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Glu  
100 105 110

Ser Glu Gln Ala Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu  
115 120 125

Gln Ala Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala  
130 135 140

Pro Glu Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu  
145 150 155 160

Pro Leu Pro Glu Ala Pro Glu Glu Ser Glu Gln Ala Pro Glu Pro Leu  
165 170 175

Pro Glu Ala Pro Glu Glu Phe Asp Gln Ala Pro Met Pro Leu Pro Ala  
180 185 190

Ala Pro Glu Asp Phe Asp Gln Pro Ala Met Pro Leu Pro Pro Ala Pro  
195 200 205

Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Gln Ala Pro Glu Glu  
210 215 220

Leu Glu Gln Ala Pro Ala Ser Thr Pro Arg Arg Arg Ser Arg Arg Cys  
225 230 235 240

Leu Arg Glu Lys Leu Thr Gln Glu Val Asn Leu Arg Arg Ile  
245 250

<210> 127

<211> 236

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1) .. (234)

<400> 127

cgc gga gag ggg gag act gag aga ggg cag aat gag gag act cac gca 48  
Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala  
1 5 10 15

acg aac aag tcc tca ggc gtc gcc agt ttg gag gca cca gcg tcg ttc 96  
 Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe  
                   20                                  25                                  30

gcg cag gag ggc gac gga ggg cgg aga gaa gaa gca agc caa gca aaa 144  
 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys  
                   35                                  40                                  45

atg ggg acg tct ccc ccg tcg aat cag gtg atc aac gtt gta gac gaa 192  
 Met Gly Thr Ser Pro Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu  
                   50                                  55                                  60

gac gag gag gac gac gag gaa gca gag gcg cta gag gct ccc.gg 236  
 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Leu Glu Ala Pro  
                   65                                  70                                  75

<210> 128

<211> 78

<212> PRT

<213> Toxoplasma gondii

<400> 128

Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala  
           1                                  5                                  10                                  15

Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe  
                   20                                  25                                  30

Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys  
                   35                                  40                                  45

Met Gly Thr Ser Pro Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu  
                   50                                  55                                  60

Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Leu Glu Ala Pro  
                   65                                  70                                  75

<210> 129

<211> 569

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(567)

&lt;400&gt; 129

```

cga tct cgt ttt ggt cct gag caa ttc gct att tcg gat gtc tca ggc      48
Arg Ser Arg Phe Gly Pro Glu Gln Phe Ala Ile Ser Asp Val Ser Gly
  1              5              10              15

aca ctt gtt aat gca agc tgg ctt ggt gcc tct gca ggg gag act atc      96
Thr Leu Val Asn Ala Ser Trp Leu Gly Ala Ser Ala Gly Glu Thr Ile
              20              25              30

gct gat tca agg gct tta agg cgt gac cta tca ttc cca ctg tct agt      144
Ala Asp Ser Arg Ala Leu Arg Arg Asp Leu Ser Phe Pro Leu Ser Ser
              35              40              45

cgt caa ctg cga gaa cgt ggc ctt gct tct caa gat tcc tca ctt tca      192
Arg Gln Leu Arg Glu Arg Gly Leu Ala Ser Gln Asp Ser Ser Leu Ser
              50              55              60

agc act cca aaa ttg tcc ctg caa cac gac cac ttt gca aag act ctg      240
Ser Thr Pro Lys Leu Ser Leu Gln His Asp His Phe Ala Lys Thr Leu
              65              70              75              80

gta aaa cga aga gcg ctg tct gca acg aac tcc aca gaa cgc agc ggc      288
Val Lys Arg Arg Ala Leu Ser Ala Thr Asn Ser Thr Glu Arg Ser Gly
              85              90              95

aaa cca gtt cgt tgc ttt act gaa acc agc gtg agg tta ggt gca cct      336
Lys Pro Val Arg Cys Phe Thr Glu Thr Ser Val Arg Leu Gly Ala Pro
              100              105              110

act caa ccg gta atg gag gaa atg cct ttg gga gaa gga gag gta aat      384
Thr Gln Pro Val Met Glu Glu Met Pro Leu Gly Glu Gly Glu Val Asn
              115              120              125

ctg gtc tcc gaa cac gac gat tat gca gaa tcc acc agt cat ctg gat      432
Leu Val Ser Glu His Asp Asp Tyr Ala Glu Ser Thr Ser His Leu Asp
              130              135              140

acg gtg aat ggg aga gaa aga aga gag gaa agg cat tac gcg gag acg      480
Thr Val Asn Gly Arg Glu Arg Arg Glu Glu Arg His Tyr Ala Glu Thr
              145              150              155              160

gag gcg aca gac gaa ttc aaa tcc gca atg cac cac gtg acg tcg ccc      528
Glu Ala Thr Asp Glu Phe Lys Ser Ala Met His His Val Thr Ser Pro
              165              170              175

gga ggg gta ccc gca acg aaa aag gtg gtg tgg aag atc cg      569
Gly Gly Val Pro Ala Thr Lys Lys Val Val Trp Lys Ile

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180

185

&lt;210&gt; 130

&lt;211&gt; 189

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 130

Arg Ser Arg Phe Gly Pro Glu Gln Phe Ala Ile Ser Asp Val Ser Gly  
 1 5 10 15

Thr Leu Val Asn Ala Ser Trp Leu Gly Ala Ser Ala Gly Glu Thr Ile  
 20 25 30

Ala Asp Ser Arg Ala Leu Arg Arg Asp Leu Ser Phe Pro Leu Ser Ser  
 35 40 45

Arg Gln Leu Arg Glu Arg Gly Leu Ala Ser Gln Asp Ser Ser Leu Ser  
 50 55 60

Ser Thr Pro Lys Leu Ser Leu Gln His Asp His Phe Ala Lys Thr Leu  
 65 70 75 80

Val Lys Arg Arg Ala Leu Ser Ala Thr Asn Ser Thr Glu Arg Ser Gly  
 85 90 95

Lys Pro Val Arg Cys Phe Thr Glu Thr Ser Val Arg Leu Gly Ala Pro  
 100 105 110

Thr Gln Pro Val Met Glu Glu Met Pro Leu Gly Glu Gly Glu Val Asn  
 115 120 125

Leu Val Ser Glu His Asp Asp Tyr Ala Glu Ser Thr Ser His Leu Asp  
 130 135 140

Thr Val Asn Gly Arg Glu Arg Arg Glu Glu Arg His Tyr Ala Glu Thr  
 145 150 155 160

Glu Ala Thr Asp Glu Phe Lys Ser Ala Met His His Val Thr Ser Pro  
 165 170 175

Gly Gly Val Pro Ala Thr Lys Lys Val Val Trp Lys Ile  
 180 185

&lt;210&gt; 131

&lt;211&gt; 232

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 131

cggcgactca gatgggagtg agaaagatgc aaacaggtgc tgaaaaaaca ccacttaata 60  
 gaggagacaa accccggtgg agaaagcgaa acgagactgg aacggcaacg aaatagagaa 120  
 gacacagccc caaactcccg acagcgtggt gctctgtcgg gcaggcaggc caagctggca 180  
 agccgctagc atgccacgtg ctgtactgct ggcccgaac tacagtgcgc ac 232

&lt;210&gt; 132

&lt;211&gt; 276

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(276)

&lt;400&gt; 132

ccc gga att ccg gct ccg ggt cgc aaa gcg atc cat ttg ata aaa gac 48  
 Pro Gly Ile Pro Ala Pro Gly Arg Lys Ala Ile His Leu Ile Lys Asp  
 1 5 10 15

tgc gtt ttc tgc ctt ggg gaa ctc ttc ttg aat ggc acg aga ggc cac 96  
 Cys Val Phe Cys Leu Gly Glu Leu Phe Leu Asn Gly Thr Arg Gly His  
 20 25 30

aga cag aga gag agg gag gga aag cca aag aag caa aca ggc tcg gaa 144  
 Arg Gln Arg Glu Arg Glu Gly Lys Pro Lys Lys Gln Thr Gly Ser Glu  
 35 40 45

gcg ccc aga ata cag gca gcc tct ccg aag tca ctc acc ttg tac gat 192  
 Ala Pro Arg Ile Gln Ala Ala Ser Pro Lys Ser Leu Thr Leu Tyr Asp  
 50 55 60

ctt gtg cac agt gat gta ggg cgc atg cag aac gac gcc tcc aac atg 240  
 Leu Val His Ser Asp Val Gly Arg Met Gln Asn Asp Ala Ser Asn Met  
 65 70 75 80

aat att ctc ctc ggc caa ggc cgc cgc caa gta gcg 276  
 Asn Ile Leu Leu Gly Gln Gly Arg Arg Gln Val Ala  
 85 90

&lt;210&gt; 133

&lt;211&gt; 92

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 133

Pro Gly Ile Pro Ala Pro Gly Arg Lys Ala Ile His Leu Ile Lys Asp  
 1 5 10 15

Cys Val Phe Cys Leu Gly Glu Leu Phe Leu Asn Gly Thr Arg Gly His  
 20 25 30

Arg Gln Arg Glu Arg Glu Gly Lys Pro Lys Lys Gln Thr Gly Ser Glu  
 35 40 45

Ala Pro Arg Ile Gln Ala Ala Ser Pro Lys Ser Leu Thr Leu Tyr Asp  
 50 55 60

Leu Val His Ser Asp Val Gly Arg Met Gln Asn Asp Ala Ser Asn Met  
 65 70 75 80

Asn Ile Leu Leu Gly Gln Gly Arg Arg Gln Val Ala  
 85 90

&lt;210&gt; 134

&lt;211&gt; 309

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(309)

&lt;400&gt; 134

cgc gga cac act gga gag agg tgg tcg gac agg gag gga gaa tcc gag 48  
 Arg Gly His Thr Gly Glu Arg Trp Ser Asp Arg Glu Gly Glu Ser Glu  
 1 5 10 15

atg tgc agt gga gga caa atg gaa aag aga gag agc cga cgc gtt tct 96  
 Met Cys Ser Gly Gly Gln Met Glu Lys Arg Glu Ser Arg Arg Val Ser  
 20 25 30

ttt gcg gat gaa gag atg cgg aat ccg aca gaa aac ctg aag gta gat 144  
 Phe Ala Asp Glu Glu Met Arg Asn Pro Thr Glu Asn Leu Lys Val Asp  
 35 40 45

gcc aac tgt gtg ctc gaa ggt ctg tcc acc tca gtg tgt gcg agg cgg 192

Ala Asn Cys Val Leu Glu Gly Leu Ser Thr Ser Val Cys Ala Arg Arg  
 50 55 60

ctg aag agg caa aag cga act gca ggt cag tct ggc ttc ctc gca ata 240  
 Leu Lys Arg Gln Lys Arg Thr Ala Gly Gln Ser Gly Phe Leu Ala Ile  
 65 70 75 80

cga aac gtc caa ggc acc gcg acc gcc cta aaa cac cct gat tcc aca 288  
 Arg Asn Val Gln Gly Thr Ala Thr Ala Leu Lys His Pro Asp Ser Thr  
 85 90 95

gga cga cgg tct tgg gat ccg 309  
 Gly Arg Arg Ser Trp Asp Pro  
 100

<210> 135  
 <211> 103  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 135  
 Arg Gly His Thr Gly Glu Arg Trp Ser Asp Arg Glu Gly Glu Ser Glu  
 1 5 10 15

Met Cys Ser Gly Gly Gln Met Glu Lys Arg Glu Ser Arg Arg Val Ser  
 20 25 30

Phe Ala Asp Glu Glu Met Arg Asn Pro Thr Glu Asn Leu Lys Val Asp  
 35 40 45

Ala Asn Cys Val Leu Glu Gly Leu Ser Thr Ser Val Cys Ala Arg Arg  
 50 55 60

Leu Lys Arg Gln Lys Arg Thr Ala Gly Gln Ser Gly Phe Leu Ala Ile  
 65 70 75 80

Arg Asn Val Gln Gly Thr Ala Thr Ala Leu Lys His Pro Asp Ser Thr  
 85 90 95

Gly Arg Arg Ser Trp Asp Pro  
 100

<210> 136  
 <211> 534  
 <212> DNA  
 <213> Toxoplasma gondii



&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(534)

&lt;400&gt; 136

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cgg atc gag gct gaa atc gct agg cag aag gag cgg gaa gcc aaa ctg      48
Arg Ile Glu Ala Glu Ile Ala Arg Gln Lys Glu Arg Glu Ala Lys Leu
   1               5               10               15

cgt cgc agg ctt gct gcg gtc gtg gcc tca atg ctg gta gca gcg agc      96
Arg Arg Arg Leu Ala Ala Val Val Ala Ser Met Leu Val Ala Ala Ser
           20               25               30

ctc tac ggc ttg aac tcg ttc ctc cac ggt tct gac aag gag att tct      144
Leu Tyr Gly Leu Asn Ser Phe Leu His Gly Ser Asp Lys Glu Ile Ser
           35               40               45

tcg atg cca tcc tct atc gac aaa aaa cca gat tcc ccc ttt gcc gca      192
Ser Met Pro Ser Ser Ile Asp Lys Lys Pro Asp Ser Pro Phe Ala Ala
           50               55               60

cag ctg ggc acc tcg ctc gag tca gaa att ggt ata ccc gaa gaa aaa      240
Gln Leu Gly Thr Ser Leu Glu Ser Glu Ile Gly Ile Pro Glu Glu Lys
           65               70               75               80

gca att cct gag gcg gcc gac ata agc agt ttt att gag aat ctt tcc      288
Ala Ile Pro Glu Ala Ala Asp Ile Ser Ser Phe Ile Glu Asn Leu Ser
           85               90               95

gcg acg gtg gca ggc aat tct gtg caa gcc cag agc atc ggc ttt gtg      336
Ala Thr Val Ala Gly Asn Ser Val Ala Gln Ser Ile Gly Phe Val
           100              105              110

ttg aca gtt gtt gta ctt ggt ctt gtc gcc ttc tca ctc aag gct gct      384
Leu Thr Val Val Val Leu Gly Leu Val Ala Phe Ser Leu Lys Ala Ala
           115              120              125

cga cgt tcc tcg cca aga gag gag cag gca ttc agc ctg ccg gca cac      432
Arg Arg Ser Ser Pro Arg Glu Glu Gln Ala Phe Ser Leu Pro Ala His
           130              135              140

ccg cct cgc gag gaa aaa tca aaa tac ctg ctg aag ccg ccc cag cag      480
Pro Pro Arg Glu Glu Lys Ser Lys Tyr Leu Leu Lys Pro Pro Gln Gln
           145              150              155              160

ccc aag ccc agg cgg ctc aaa agg cag ctc cgc aag tac cga caa agg      528
Pro Lys Pro Arg Arg Leu Lys Arg Gln Leu Arg Lys Tyr Arg Gln Arg

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165

170

175

gtg ctg  
Val Leu

534

&lt;210&gt; 137

&lt;211&gt; 178

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 137

Arg Ile Glu Ala Glu Ile Ala Arg Gln Lys Glu Arg Glu Ala Lys Leu  
1 5 10 15

Arg Arg Arg Leu Ala Ala Val Val Ala Ser Met Leu Val Ala Ala Ser  
20 25 30

Leu Tyr Gly Leu Asn Ser Phe Leu His Gly Ser Asp Lys Glu Ile Ser  
35 40 45

Ser Met Pro Ser Ser Ile Asp Lys Lys Pro Asp Ser Pro Phe Ala Ala  
50 55 60

Gln Leu Gly Thr Ser Leu Glu Ser Glu Ile Gly Ile Pro Glu Glu Lys  
65 70 75 80

Ala Ile Pro Glu Ala Ala Asp Ile Ser Ser Phe Ile Glu Asn Leu Ser  
85 90 95

Ala Thr Val Ala Gly Asn Ser Val Gln Ala Gln Ser Ile Gly Phe Val  
100 105 110

Leu Thr Val Val Val Leu Gly Leu Val Ala Phe Ser Leu Lys Ala Ala  
115 120 125

Arg Arg Ser Ser Pro Arg Glu Glu Gln Ala Phe Ser Leu Pro Ala His  
130 135 140

Pro Pro Arg Glu Glu Lys Ser Lys Tyr Leu Leu Lys Pro Pro Gln Gln  
145 150 155 160

Pro Lys Pro Arg Arg Leu Lys Arg Gln Leu Arg Lys Tyr Arg Gln Arg  
165 170 175

Val Leu

<210> 138  
 <211> 423  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <223> At locations 6, 23 and 34, N = unknown

<400> 138  
 tgggtgntggt gaggccgcat cgnagagggga gacntgctag agccaggggc agagccgcag 60  
 gtccggtgga ggatgttggt gagccccct cgggagtgga agacctgccg cagccagagg 120  
 cagaggcgca agtaccgacc aagggtgttg accatgccgc gtcgggaggg caggacatcg 180  
 tggagccaga ggcagagccg cagggactgg tggctggcgc tggtagggcc acatcgagg 240  
 gggaggacct gctagagcca ggggcagcgc cgcagggtcc ggtgaaggat attatcgag 300  
 cggcgtcggg agaggaagaa ctgctggagc cagaggcaaa gccgcagggt tctatcgag 360  
 atgttgatga ggcagcgctg ggaggggagg acctgctaga gccagaggca gggatcgag 420  
 tcc 423

<210> 139  
 <211> 327  
 <212> DNA  
 <213> *Toxoplasma gondii*

<220>  
 <221> CDS  
 <222> (1)..(327)

<400> 139  
 cgc tct caa tca aca aag cca ccc gcg cct tca gac gta gag tct aca 48  
 Arg Ser Gln Ser Thr Lys Pro Pro Ala Pro Ser Asp Val Glu Asp Thr  
 1 5 10 15  
 ggc tct tct gac aac ccg ggt gac aat gtg aca gag gac aca act gag 96  
 Gly Ser Ser Asp Asn Pro Gly Asp Asn Val Thr Glu Asp Thr Thr Glu  
 20 25 30  
 agt cca tca cag ggc acc gac ggt tca gca tcc gga ccc ggg tct act 144  
 Ser Pro Ser Gln Gly Thr Asp Gly Ser Ala Ser Gly Pro Gly Ser Thr  
 35 40 45

cat ccg gaa aac gac gcg ggg gaa cat gag gat ggc gcg tca ctg ggg 192  
 His Pro Glu Asn Asp Ala Gly Glu His Glu Asp Gly Ala Ser Leu Gly  
           50                          55                          60

caa gac cag caa gag cgc atg gat aaa tct tcc cta ggc aaa gaa aca 240  
 Gln Asp Gln Gln Glu Arg Met Asp Lys Ser Ser Leu Gly Lys Glu Thr  
           65                          70                          75                          80

ccc atg ctc gat cag gga aat tcg tca cca gca aca acg ggg tcc ggt 288  
 Pro Met Leu Asp Gln Gly Asn Ser Ser Pro Ala Thr Thr Gly Ser Gly  
                                   85                          90                          95

gcc cat gaa aaa aac gag agc gtg tca gga gtt cca gcg 327  
 Ala His Glu Lys Asn Glu Ser Val Ser Gly Val Pro Ala  
                           100                          105

<210> 140

<211> 109

<212> PRT

<213> Toxoplasma gondii

<400> 140

Arg Ser Gln Ser Thr Lys Pro Pro Ala Pro Ser Asp Val Glu Asp Thr  
       1                          5                          10                          15

Gly Ser Ser Asp Asn Pro Gly Asp Asn Val Thr Glu Asp Thr Thr Glu  
                           20                          25                          30

Ser Pro Ser Gln Gly Thr Asp Gly Ser Ala Ser Gly Pro Gly Ser Thr  
           35                          40                          45

His Pro Glu Asn Asp Ala Gly Glu His Glu Asp Gly Ala Ser Leu Gly  
           50                          55                          60

Gln Asp Gln Gln Glu Arg Met Asp Lys Ser Ser Leu Gly Lys Glu Thr  
           65                          70                          75                          80

Pro Met Leu Asp Gln Gly Asn Ser Ser Pro Ala Thr Thr Gly Ser Gly  
                           85                          90                          95

Ala His Glu Lys Asn Glu Ser Val Ser Gly Val Pro Ala  
                           100                          105

<210> 141

<211> 444

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(444)

&lt;400&gt; 141

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ccg gcg cga act ggc gac gcg cag cct gag ggc aga gag ggg cac agc   48
Pro Ala Arg Thr Gly Asp Ala Gln Pro Glu Gly Arg Glu Gly His Ser
   1             5             10             15

cca ctg gaa gac gaa ggg aga gat gcg ttt gga aga cgc gct gcg gaa   96
Pro Leu Glu Asp Glu Gly Arg Asp Ala Phe Gly Arg Arg Ala Ala Glu
           20           25           30

gac gag aga aac aga gga aat ccg aat gcg gct ggc gag act tcc caa   144
Asp Glu Arg Asn Arg Gly Asn Pro Asn Ala Ala Gly Glu Thr Ser Gln
           35           40           45

gac gag gca gag aac gcg caa gcg tcc ctg cgg ttc gct gcg aga gag   192
Asp Glu Ala Glu Asn Ala Gln Ala Ser Leu Arg Phe Ala Ala Arg Glu
           50           55           60

aaa cct ctc gaa gtc ctc aga ttc cga gaa gac act gca gac act ctg   240
Lys Pro Leu Glu Val Leu Arg Phe Arg Glu Asp Thr Ala Asp Thr Leu
           65           70           75           80

acg tat gca gac tat cca aac agc gtg gag ttc aca ccc gca gac atg   288
Thr Tyr Ala Asp Tyr Pro Asn Ser Val Glu Phe Thr Pro Ala Asp Met
           85           90           95

ccg aat gcg aag gac cag acg cct ctg cat gca aag tac aat cac ttt   336
Pro Asn Ala Lys Asp Gln Thr Pro Leu His Ala Lys Tyr Asn His Phe
           100          105          110

tgc gcc tac tca tgc tgg ctg acc tcg cgc ttc aac cca gac aac cca   384
Cys Ala Tyr Ser Cys Trp Leu Thr Ser Arg Phe Asn Pro Asp Asn Pro
           115          120          125

aac agc cac tgt gga aaa gga aaa aac gag aaa cgc cga ttc gac gac   432
Asn Ser His Cys Gly Lys Gly Lys Asn Glu Lys Arg Arg Phe Asp Asp
           130          135          140

gac tac gat ccg   444
Asp Tyr Asp Pro
145

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&lt;210&gt; 142

&lt;211&gt; 148

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 142

Pro Ala Arg Thr Gly Asp Ala Gln Pro Glu Gly Arg Glu Gly His Ser  
 1 5 10 15

Pro Leu Glu Asp Glu Gly Arg Asp Ala Phe Gly Arg Arg Ala Ala Glu  
 20 25 30

Asp Glu Arg Asn Arg Gly Asn Pro Asn Ala Ala Gly Glu Thr Ser Gln  
 35 40 45

Asp Glu Ala Glu Asn Ala Gln Ala Ser Leu Arg Phe Ala Ala Arg Glu  
 50 55 60

Lys Pro Leu Glu Val Leu Arg Phe Arg Glu Asp Thr Ala Asp Thr Leu  
 65 70 75 80

Thr Tyr Ala Asp Tyr Pro Asn Ser Val Glu Phe Thr Pro Ala Asp Met  
 85 90 95

Pro Asn Ala Lys Asp Gln Thr Pro Leu His Ala Lys Tyr Asn His Phe  
 100 105 110

Cys Ala Tyr Ser Cys Trp Leu Thr Ser Arg Phe Asn Pro Asp Asn Pro  
 115 120 125

Asn Ser His Cys Gly Lys Gly Lys Asn Glu Lys Arg Arg Phe Asp Asp  
 130 135 140

Asp Tyr Asp Pro  
 145

&lt;210&gt; 143

&lt;211&gt; 928

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(57)

&lt;400&gt; 143

cgg gat ccc gag cgg gac ctc ccg gtg tcc tcg act gct cat aca cca 48  
 Arg Asp Pro Glu Arg Asp Leu Pro Val Ser Ser Thr Ala His Thr Pro  
 1 5 10 15

gag gag gat tgattcaccg cagaacttaa cccgtgggac gcagcctcca 97  
 Glu Glu Asp

cagagtctgt ggcggacgaa ggaaatgcag gaacagagag acctggaccg aaaaatggca 157  
 tggacgatgg gtgtccacgg gagaagacgg atactcgtgg aaagccgggg gaaagcgacg 217  
 gagggaaatg cgcgacaagc tggaaaagcg agctcacaac gacgaaacac gcactgtgca 277  
 tccgaacgac aatgacctgt ccttagtaga cgagaggggg taggcaacaa ttcctcagaa 337  
 gtccaccagc gaccggacag cgaccgcggc agcacgttga gggaggtctt actagcggcc 397  
 gagactcagg acaacaggag ccctctaccg ccatgcgaca cacgcagaac aacgctttag 457  
 attaaggtcg aaaaaggaaa cctcaacgca gaacgagtca cttctttccc aaaaagtgc 517  
 tgtggaaaaa cagcgcatgc ggggctgggt gactcgaaaa tctgggaacg cgtctggcag 577  
 gcatcctgcc cgaacccgat accagagaaa cggaacccgt actggctgga attcaacagt 637  
 acggacaaaa aaccacccgt gtaaagtgga caaaagccga caatggaaca actacttggg 697  
 agaaagcaaa tgctgcgttc accaggccag tgtcacgccg cgtcgtaaag aaggcggctc 757  
 agcgttgccg gtgtgcctgg cgttgtcgga cggcttccgt gtgtacccaa cagaaaagct 817  
 tttaactct tactatcttc atttgccac gatttctttt ttgcctatct actgtaccta 877  
 gccacgtcgc cattctaagg aagttgtccc gttgcaacgc agaacgcgga g 928

<210> 144

<211> 19

<212> PRT

<213> *Toxoplasma gondii*

<400> 144

Arg Asp Pro Glu Arg Asp Leu Pro Val Ser Ser Thr Ala His Thr Pro  
 1 5 10 15

Glu Glu Asp

<210> 145  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 145  
cgcttcttgt gtcacctg

18

<210> 146  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<400> 146  
gcaccttggt ctctctcttc gcc

23

<210> 147  
<211> 18  
<212> DNA  
<213> Toxoplasma gondii

<400> 147  
cgaggagacg gtgggagc

18

<210> 148  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 148  
tgcccaagat gccgatctct g

21

<210> 149  
<211> 20  
<212> DNA



<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 149

tctcccccac cgacgaaaac

20

<210> 150

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 150

gctcatttcc tccgcaattt gg

22

<210> 151

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 151

agctggcaga aataccaaag ctc

23

<210> 152

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 152

tgctcggaat actgggcatg

20

<210> 153

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 153

actggagtgg aaagtctggt ttg

24

<210> 154

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 154

gacgcagaga agaaagaaga gcc

23

<210> 155

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 155

tcctaaaactg tctcgtctcc cc

22

<210> 156

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 156  
tctggatacgcggttccttt g 21

<210> 157  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 157  
gacatctacc tgtgagtgaa ccagg 25

<210> 158  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 158  
gtcaaaacct tgccagcatc tc 22

<210> 159  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 159  
tccgactgaa tgactacctc tttc 24

<210> 160  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 160

tccgaccaag tcctcagtga ac

22

<210> 161

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 161

tgggcatttc ctggaagagg

20

<210> 162

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 162

gaatccatct cgtgcaaacg g

21

<210> 163

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 163

caagacacag ggaaacgttg g

21

<210> 164  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 164  
gaaagaatcg cacctcctct cc

22

<210> 165  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 165  
tttgagtcta accgccgtat gtc

23

<210> 166  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 166  
tcagacgatt ctcccattgt acg

23

<210> 167  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 167  
tcgacttggg tccgattggt ag

22

<210> 168  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 168  
gatcttttgc gtgactttgt ctgc

24

<210> 169  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 169  
gaagatgctt gtcttggtcg gttc

24

<210> 170  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 170  
gaggggtttc cttctttatt gcc

23

<210> 171  
<211> 20  
<212> DNA  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 171

tgttggacat cccgagcatc

20

&lt;210&gt; 172

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 172

ggtccttggt tttcaggcgg

20

&lt;210&gt; 173

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 173

tcgtgcagac agtgaagcaa tg

22

&lt;210&gt; 174

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 174

ttttgtcagc acagagtggc g

21

&lt;210&gt; 175

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 175

cgcaagtgag ttttgcttt acc

23

<210> 176

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 176

cctggaagag atatgcagac ac

22

<210> 177

<211> 22

<212> DNA

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<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 177

tcaccgttcg ctcttctttc tc

22

<210> 178

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 178



cgactgaagc atggattgcc

20

&lt;210&gt; 179

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 179

acatattcct gaggaggagt tccc

24

&lt;210&gt; 180

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 180

aacacacctc cgacgacacc ac

22

&lt;210&gt; 181

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 181

ctcggcttct ccacatacaa gg

22

&lt;210&gt; 182

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 182

ggatctaggc atttgggttt cac

23

<210> 183

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 183

atcgaagaag ctgaagcgga g

21

<210> 184

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 184

gtgcttgtct ctgacgaaac cc

22

<210> 185

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 185

tatcattgta tcccgtcgtc cc

22

<210> 186

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 186

tgatgcctgg attgcacaa c

21

<210> 187

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 187

cggatcgctc tgagtctctt tg

22

<210> 188

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 188

atcctgtgtc ttctcttcga ccc

23

<210> 189

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 189

gatcgctctg agtctctttg

20

<210> 190  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 190  
acgtgaggga gaagaagaga gtgc

24

<210> 191  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 191  
ttcatcgctcg cctctgatgt cc

22

<210> 192  
<211> 23  
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<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 192  
tgtagacagc gtttagggag tgc

23

<210> 193  
<211> 22  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: Synthetic

## Primer

&lt;400&gt; 193

gtccttggaagtgacagaagc ag

22

&lt;210&gt; 194

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 194

aagcgaggaa aaggaggtgt c

21

&lt;210&gt; 195

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 195

cgggaagggtt ggtgatgtct gtg

23

&lt;210&gt; 196

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 196

cccgaagact ttgacctg

18

&lt;210&gt; 197

&lt;211&gt; 18

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 197

agtggcatag gaggtctgg

18

<210> 198

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 198

gcaccttcaa tgccacaggt atc

23

<210> 199

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 199

tcgtgtgctt ctgcttctc tg

22

<210> 200

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 200

cactgtcgat cagaagaagg cttac

25

<210> 201  
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<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 201  
gctccgtggg cacatttttg

20

<210> 202  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 202  
cagtttacga ggtacaaggc aacag

25

<210> 203  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 203  
gattgcgtgg gcagtgtaga ag

22

<210> 204  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 204

tgtttgtttc cccagtcaac gac

23

&lt;210&gt; 205

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 205

cggaagaggt tgttggactc cttc

24

&lt;210&gt; 206

&lt;211&gt; 25

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 206

caaccgagag agaagagagg aacag

25

&lt;210&gt; 207

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 207

tggggagaac agcagacatc ag

22

&lt;210&gt; 208

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence



<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 208

ggatgaacac tggatcatca tg

22

<210> 209

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 209

cgacttggtc cgctc

15

<210> 210

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 210

cggcggcaac aaatgggc

18

<210> 211

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 211

gtccgagata tgaggatgcg ac

22

<210> 212  
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<212> DNA  
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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 212  
tcagagcacc attgttgcca c

21

<210> 213  
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Primer

<400> 213  
tttgacgctc aagtggaggc tg

22

<210> 214  
<211> 19  
<212> DNA  
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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 214  
gcctgcaacg ctcgatggc

19

<210> 215  
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Primer

<400> 215  
cttcttgact accttcacgt ctg

23

<210> 216  
<211> 18  
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Primer

<400> 216  
aaggacaagc ctggtttg

18

<210> 217  
<211> 18  
<212> DNA  
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Primer

<400> 217  
tttgcccttc gcacaatc

18

<210> 218  
<211> 22  
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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 218  
ccagttttgc cagaggaaga cc

22

<210> 219  
<211> 22  
<212> DNA  
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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 219

atccgtcaat gcaggtttca tc

22

<210> 220

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<212> DNA

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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 220

agacaccaga gacagcagca gtc

23

<210> 221

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 221

acttcgcccg acaatcgctt tcc

23

<210> 222

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 222

cgatcctccc gagggacc

18

<210> 223

<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 223

gcctttacgc attcaagtcg tg

22

<210> 224

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 224

ttcagcgggt ctttcctcac

20

<210> 225

<211> 22

<212> DNA

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<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 225

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22

<210> 226

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 226

aactttcttgc acttggtccc g

21

<210> 227

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 227

aagcgaggaa aaggaggtgt ctc

23

<210> 228

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 228

ggaaggttgg tgatgtctgt g

21

<210> 229

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 229

tccccccagga attggtgaaa cag

23

<210> 230

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 230

actaccgaca acgtctcagt ccttc

25

<210> 231

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 231

cgtgcgtctg tgaggaaaag tg

22

<210> 232

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 232

ttgttgctcg tgttgcaggt gc

22

<210> 233

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 233

ttgttctcga acccgagag

20

<210> 234

<211> 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 234

tggcaagaga ccgaatcgtg

20

&lt;210&gt; 235

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 235

aaacttggca aaggggaacg

20

&lt;210&gt; 236

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 236

tgctgtggag aatgatggct g

21

&lt;210&gt; 237

&lt;211&gt; 17

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 237

tttccgacga agctgcc

17



<210> 238

<211> 19

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 238

gactccaacg aaagcctcg

19

<210> 239

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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 239

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20

<210> 240

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 240

aagcagagga gagacgagac gaag

24

<210> 241

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

## Primer

&lt;400&gt; 241

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24

&lt;210&gt; 242

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 242

gcaaaaagcgg actcgattct attg

24

&lt;210&gt; 243

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 243

tgtggcagag caaaaggctc

20

&lt;210&gt; 244

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 244

ctgtggatgc tcctttgcga ct

22

&lt;210&gt; 245

&lt;211&gt; 20

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 245

cgaggcaccc gaagaatttg

20

<210> 246

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 246

cttctcaggt tcacttcctg cg

22

<210> 247

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 247

tcacgcaacg aacaagtcct c

21

<210> 248

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 248

cccatttttg cttggcttgc

20

<210> 249  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 249  
agcggcaaac cagttcgttg

20

<210> 250  
<211> 20  
<212> DNA  
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 250  
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20

<210> 251  
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<212> DNA  
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<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 251  
cggcgactca gatggg

16

<210> 252  
<211> 23  
<212> DNA  
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<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 252  
ggggctgtgt cttctctatt tcg 23

<210> 253  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 253  
aagcaaacag gctcggaagc 20

<210> 254  
<211> 20  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 254  
tcatgttgga ggcgtcgttc 20

<210> 255  
<211> 21  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 255  
tgtgcagtgg aggacaaatg g 21

<210> 256  
<211> 21  
<212> DNA  
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<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 256

gaatcagggt gttttagggc g

21

<210> 257

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 257

attctgtgca agcccagag

19

<210> 258

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 258

cgaccaaggg tggtgaccat

20

<210> 259

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 259

ctaggcaaag aaacacccat gc

22

<210> 260

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 260

cgctggaact cctgacac

18

<210> 261

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 261

acgaaggag agatgcgttt g

21

<210> 262

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 262

tggtgtttg ggttgtctgg

20

<210> 263

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 263

tcaccgcaga acttaaccg

20

&lt;210&gt; 264

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 264

ctcgcttttc cagcttgctg

20

&lt;210&gt; 265

&lt;211&gt; 311

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(288)

&lt;400&gt; 265

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Arg	Asp	Pro	Ala	Ala	Pro	Asn	Ser	Thr	Gln	Ala	Val	Ala	Ala	Ala	Arg	
1				5					10				15			

acc	gtg	gta	gtg	atg	aaa	acc	gac	gca	gaa	gtg	tcc	ggg	gac	aac	ctc	96
Thr	Val	Val	Val	Met	Lys	Thr	Asp	Ala	Glu	Val	Ser	Gly	Asp	Asn	Leu	
			20					25					30			

agt	cag	ccg	ggg	agg	cgt	ccg	ccg	tcg	cca	aag	ccg	caa	acg	acg	aag	144
Ser	Gln	Pro	Gly	Arg	Arg	Pro	Pro	Ser	Pro	Lys	Pro	Gln	Thr	Thr	Lys	
		35				40						45				

ttt	ccg	ccg	aga	gag	tca	cca	gac	cgc	agg	ggg	acg	agg	cgg	aga	act	192
Phe	Pro	Arg	Arg	Glu	Ser	Pro	Asp	Arg	Arg	Gly	Thr	Arg	Arg	Arg	Thr	
		50				55				60						

gaa	agc	cga	ggc	gct	gtt	agc	agg	gta	tgg	cca	ggg	gaa	aac	cag	cga	240
Glu	Ser	Arg	Gly	Ala	Val	Ser	Arg	Val	Trp	Pro	Gly	Glu	Asn	Gln	Arg	
	65				70				75				80			

aga	cgg	tct	gcc	gtc	gac	gat	tcg	ata	ccg	gct	aac	ccc	atc	gct	ttg	288
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Arg Arg Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ile Ala Leu  
                   85                  90                  95

aacgcgtggc gccctgcgat ccg

311

<210> 266

<211> 96

<212> PRT

<213> Toxoplasma gondii

<400> 266

Arg Asp Pro Ala Ala Pro Asn Ser Thr Gln Ala Val Ala Ala Ala Arg  
   1                  5                  10                  15

Thr Val Val Val Met Lys Thr Asp Ala Glu Val Ser Gly Asp Asn Leu  
                   20                  25                  30

Ser Gln Pro Gly Arg Arg Pro Pro Ser Pro Lys Pro Gln Thr Thr Lys  
                   35                  40                  45

Phe Pro Arg Arg Glu Ser Pro Asp Arg Arg Gly Thr Arg Arg Arg Thr  
                   50                  55                  60

Glu Ser Arg Gly Ala Val Ser Arg Val Trp Pro Gly Glu Asn Gln Arg  
   65                  70                  75                  80

Arg Arg Ser Ala Val Asp Asp Ser Ile Pro Ala Asn Pro Ile Ala Leu  
                   85                  90                  95

<210> 267

<211> 303

<212> DNA

<213> Toxoplasma gondii

<220>

<221> CDS

<222> (1)..(303)

<400> 267

gac gaa gct ctt cct ctc ttt gga gca aac gat gga acc tca gtt cgg 48  
 Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Asp Gly Thr Ser Val Arg  
   1                  5                  10                  15

ctc tcc ctc gac cgc agc gtc ctg ctt gtt ctc gaa ccc gca gag ccc 96  
 Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro  
                   20                  25                  30

ctg cta tcc tct tgg ccc cac ccg ggg aga aga gac act ttt ctt gaa 144  
 Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu  
                   35                                  40                                  45

ggc gat ggc gcg ggc atc ccg tct cct tca tct cgg ccg agt cgc gcg 192  
 Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala  
                   50                                  55                                  60

gcc gac cat tac acg aga ctc tcc acg att cgg tct ctt gcc agg gat 240  
 Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp  
                   65                                  70                                  75                                  80

gga gag gtc gac tcc gag ctg gcg ggg gga ccg cag gaa aga gaa agt 288  
 Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser  
   85                                  90                                  95

gtc aga gtg gat ccg 303  
 Val Arg Val Asp Pro  
                   100

<210> 268

<211> 101

<212> PRT

<213> Toxoplasma gondii

<400> 268

Asp Glu Ala Leu Pro Leu Phe Gly Ala Asn Asp Gly Thr Ser Val Arg  
           1                                  5                                  10                                  15

Leu Ser Leu Asp Arg Ser Val Leu Leu Val Leu Glu Pro Ala Glu Pro  
                                   20                                  25                                  30

Leu Leu Ser Ser Trp Pro His Pro Gly Arg Arg Asp Thr Phe Leu Glu  
                   35                                  40                                  45

Gly Asp Gly Ala Gly Ile Pro Ser Pro Ser Ser Arg Pro Ser Arg Ala  
                   50                                  55                                  60

Ala Asp His Tyr Thr Arg Leu Ser Thr Ile Arg Ser Leu Ala Arg Asp  
                   65                                  70                                  75                                  80

Gly Glu Val Asp Ser Glu Leu Ala Gly Gly Pro Gln Glu Arg Glu Ser  
   85                                  90                                  95

Val Arg Val Asp Pro  
                   100

<210> 269  
 <211> 236  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (1)..(234)

<400> 269  
 cgc gga gag ggg gag act gag aga ggg cag aat gag gag act cac gca 48  
 Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala  
 1 5 10 15  
 acg aac aag tcc tca ggc gtc gcc agt ttg gag gca cca gcg tcg ttc 96  
 Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe  
 20 25 30  
 gcg cag gag ggc gac gga ggg cgg aga gaa gaa gca agc caa gca aaa 144  
 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys  
 35 40 45  
 atg ggg acg tct tcc ccg tcg aat cag gtg atc aac gtt gta gac gaa 192  
 Met Gly Thr Ser Ser Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu  
 50 55 60  
 gac gag gag gac gac gag gaa gca gag gcg caa gag gca ccc gg 236  
 Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Gln Glu Ala Pro  
 65 70 75

<210> 270  
 <211> 78  
 <212> PRT  
 <213> Toxoplasma gondii

<400> 270  
 Arg Gly Glu Gly Glu Thr Glu Arg Gly Gln Asn Glu Glu Thr His Ala  
 1 5 10 15  
 Thr Asn Lys Ser Ser Gly Val Ala Ser Leu Glu Ala Pro Ala Ser Phe  
 20 25 30  
 Ala Gln Glu Gly Asp Gly Gly Arg Arg Glu Glu Ala Ser Gln Ala Lys  
 35 40 45

Met Gly Thr Ser Ser Pro Ser Asn Gln Val Ile Asn Val Val Asp Glu  
 50 55 60

Asp Glu Glu Asp Asp Glu Glu Ala Glu Ala Gln Glu Ala Pro  
 65 70 75

<210> 271

<211> 423

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(423)

<400> 271

cgc gga att ccg gat cag cgt agc agt cgc agc cac act gga gtg gaa 48  
 Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu  
 1 5 10 15

agt ctg gtt ttg ccc tcc aga ggg gag gaa gag gcg aga gag gag acg 96  
 Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr  
 20 25 30

tct gca acg cgc cag atg ccg acg ctt ctc tct tcg ccg agg cct cca 144  
 Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro  
 35 40 45

ctc gcg ctg ggg ttg gga gac gag tct ccc tgc gga gag tgg gtg tcg 192  
 Leu Ala Leu Gly Leu Gly Asp Glu Ser Pro Cys Gly Glu Trp Val Ser  
 50 55 60

ccg aat gac atg gtt tct gcg ttg tcc ctc tgg gaa gca ggc gag gct 240  
 Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala  
 65 70 75 80

tgg cag ttc aag aca gcg aaa att ctt gac tct ttc gaa ggg gag acc 288  
 Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr  
 85 90 95

cca gaa ggg gag gga tgc ggc gca cag gaa aag gac agc cgc atg caa 336  
 Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Lys Asp Ser Arg Met Gln  
 100 105 110

gct ggt gcg act ccc ggt gaa cgt gga ggg gcg gtc gac gaa ggt gtg 384  
 Ala Gly Ala Thr Pro Gly Glu Arg Gly Gly Ala Val Asp Glu Gly Val  
 115 120 125

gag ctt ggc tct tct ttc ttc tct gcg tct gaa gat ccg  
 Glu Leu Gly Ser Ser Phe Phe Ser Ala Ser Glu Asp Pro  
 130 135 140

423

&lt;210&gt; 272

&lt;211&gt; 141

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 272

Arg Gly Ile Pro Asp Gln Arg Ser Ser Arg Ser His Thr Gly Val Glu  
 1 5 10 15

Ser Leu Val Leu Pro Ser Arg Gly Glu Glu Glu Ala Arg Glu Glu Thr  
 20 25 30

Ser Ala Thr Arg Gln Met Pro Thr Leu Leu Ser Ser Pro Arg Pro Pro  
 35 40 45

Leu Ala Leu Gly Leu Gly Asp Glu Ser Pro Cys Gly Glu Trp Val Ser  
 50 55 60

Pro Asn Asp Met Val Ser Ala Leu Ser Leu Trp Glu Ala Gly Glu Ala  
 65 70 75 80

Trp Gln Phe Lys Thr Ala Lys Ile Leu Asp Ser Phe Glu Gly Glu Thr  
 85 90 95

Pro Glu Gly Glu Gly Cys Gly Ala Gln Glu Lys Asp Ser Arg Met Gln  
 100 105 110

Ala Gly Ala Thr Pro Gly Glu Arg Gly Gly Ala Val Asp Glu Gly Val  
 115 120 125

Glu Leu Gly Ser Ser Phe Phe Ser Ala Ser Glu Asp Pro  
 130 135 140

&lt;210&gt; 273

&lt;211&gt; 514

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(513)

&lt;400&gt; 273

cgg gat cag gct tct atg cca ctg ccc ccg gcc ccc gaa gac ttt gac	48
Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp	
1 5 10 15	
ctg cct cct atg cca ctg ccc gaa gca ccc gaa gac ttt gac cag gct	96
Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala	
20 25 30	
cct atg cca ctg ccc gag gca ccc gaa gac ttt gac cag gct cct atg	144
Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met	
35 40 45	
cca ctg ccc gag gca ccc gaa gac ttt gac cag cct cct atg cca ctg	192
Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu	
50 55 60	
ccc gag gca ccc gaa gac ttt gac cag gct cct atg cca ctg ccc gaa	240
Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu	
65 70 75 80	
gca ccc gaa gtc ttt gac cag gct cct atg cca ctg ccc gag gca ccc	288
Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro	
85 90 95	
gaa gtc ttt gac cag gct cct atg cca ctg ccc gaa gca ccc gaa gac	336
Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp	
100 105 110	
ttt gac cag gct cct atg cca ctg ccc gaa gca ccc gaa gtc ttt gac	384
Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp	
115 120 125	
cag gct cct atg cca ctg ccc gag gca ccc gaa gac ttt gac cag gct	432
Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala	
130 135 140	
cct atg cca gtg ccc gag gca ccc gaa gac ttt gac cag gct cct gag	480
Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu	
145 150 155 160	
cca ctg ccc gag gca gcc gaa gaa ttt gat ccc g	514
Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro	
165 170	

&lt;210&gt; 274

&lt;211&gt; 171

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 274

Arg Asp Gln Ala Ser Met Pro Leu Pro Pro Ala Pro Glu Asp Phe Asp  
 1 5 10 15

Leu Pro Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala  
 20 25 30

Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met  
 35 40 45

Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Pro Pro Met Pro Leu  
 50 55 60

Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Met Pro Leu Pro Glu  
 65 70 75 80

Ala Pro Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro  
 85 90 95

Glu Val Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp  
 100 105 110

Phe Asp Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Val Phe Asp  
 115 120 125

Gln Ala Pro Met Pro Leu Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala  
 130 135 140

Pro Met Pro Val Pro Glu Ala Pro Glu Asp Phe Asp Gln Ala Pro Glu  
 145 150 155 160

Pro Leu Pro Glu Ala Ala Glu Glu Phe Asp Pro  
 165 170

&lt;210&gt; 275

&lt;211&gt; 16

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
 Primer

&lt;400&gt; 275

tgcttctcaa aagccg

16

&lt;210&gt; 276

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 276

cgattgcctg caagaagtgt g

21

&lt;210&gt; 277

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 277

acagttttct ccatttcagg

20

&lt;210&gt; 278

&lt;211&gt; 19

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 278

atatactttg cgtgggcgg

19

&lt;210&gt; 279

&lt;211&gt; 17

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence



&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 279

tcctggggttt gatgctg

17

&lt;210&gt; 280

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 280

ctgttctgaa aacggtgcgg

20

&lt;210&gt; 281

&lt;211&gt; 18

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 281

gaaaacggtg ccctaaag

18

&lt;210&gt; 282

&lt;211&gt; 1225

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(87)

&lt;400&gt; 282

ggt cga gtg tcg cag aaa aag acg ctt gtc tgt gcc cgg cgt aga caa 48  
 Gly Arg Val Ser Gln Lys Lys Thr Leu Val Cys Ala Arg Arg Arg Gln  
 1 5 10 15

tct ctt cgg cct ctc gga cga acc gag ttt tca cgg tga accttttgtg 97  
 Ser Leu Arg Pro Leu Gly Arg Thr Glu Phe Ser Arg  
                   20                                  25

cttacttttc gtctcagact gtgttggtgt tccctgcttc tcaaaagccg cccttccttc 157  
 acttcttggt cgccgattgc cttcaagaag tgtggagtgc ccttcctttt ttccgggttc 217  
 tccggaagcc tcctgtagca aaatgcgctg aaatttttga cacttctcga cgggtgtctcg 277  
 ctttaggacg gacctcatgg tctttagggc accgttttct ttcacttttt ctaggaacat 337  
 cacagttttc tccatttcag ggaacgaaca atctgcaagc gtccacttgt cctgtggctg 397  
 ctgggctcgg atgccgcctc tgtttagcaa ttgtagcagg caccggatgg caagctaggt 457  
 ccacttcact gcagtttcaa cttccaaacc aaggcatctc aatttgatc gtgttctctg 517  
 tcaacaagct gttgaacctg tcgacggagt gtcgtcccgg ctctatccc gcgttcgcaa 577  
 gccgcaccgt tttcagaaca gtgttccccg tgggtgttgaa aycgggctgc gaagcgcgag 637  
 cgtttcgttt tgtggttttt tctgggaaac gatggggatc tcttcgtgtg gcgagacgct 697  
 tgcctcctgt ttcaaggcgg tgaagtccgg aaccgttgac ttcaaggggc aggagcgagt 757  
 atactcgtgg ttgatatact ttgcgtgggc ggggtggcctc agcgggtttt tcgtcggagg 817  
 gattctggaa gacttcacgg tcacagtgtg cacgatcttg atgtgcatgg ccattgcggc 877  
 gattctctgt tttccgtcgt ggccatgttt ccacagacac cctgtcagat ggacgccgca 937  
 cgacccccgc aggctggctg ctctcttcac gcagcatcaa acccaggaag aaactcctca 997  
 gaaagggtgcg gggaaaaaac gagggaagaa gagcgctgaa gtgcaacgga aaaactgagt 1057  
 gtgtgtgcgt atgtagacaa gtgagtcttc ccagagtctg tccggattgt tgcgtggatc 1117  
 gtgtcaactg gacttctcgt tcgtcaaaga cctggtcgtc tgacccatct gctctccata 1177  
 aaaaagcttg ttaacgctcc taaaaaaaaa aaaaaaaaaa aaaaaaa 1225

<210> 283

<211> 28

<212> PRT

<213> Toxoplasma gondii

&lt;400&gt; 283

Gly Arg Val Ser Gln Lys Lys Thr Leu Val Cys Ala Arg Arg Arg Gln  
 1 5 10 15

Ser Leu Arg Pro Leu Gly Arg Thr Glu Phe Ser Arg  
 20 25

&lt;210&gt; 284

&lt;211&gt; 1225

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 284

tttttttttt tttttttttt ttttttttagg agcggttaaca agcttttttta tggagagcag 60  
 atgggtcaga cgaccaggtc tttgacgaac gagaagtcca gttgacacga tccacgcaac 120  
 aatccggacg aactctggga agactcactt gtctacatac gcacacacac tcagttttttc 180  
 cyttgcactt cagcgctctt cttccctcgt tttttccccc cacttttctg aggagttttct 240  
 tcctggggtt gatgctgctt gaagagagca gccagcctgg cgggggtcgtg cggcgctccac 300  
 tcgacagggt gtctgtggaa acatggccac gacggaaaac agagaatcgc cgcaatggcc 360  
 atgcacatca agatcgtgta cactgtgacc gtgaagtctt ccagaatccc tccgacgaaa 420  
 aaccgctga ggccaccgc ccacgcaaag tatatcaacc acgagtatac tcgctcctgc 480  
 cccttgaagt caacggttcc ggacttcacc gccttgaaac aggaggcaag cgtctcgcga 540  
 cacgaagaga tccccatcgt ttcccagaaa aaaccacaaa acgaaacgct cgcgcttcgc 600  
 agcccgtttt caacaccacg gggaacactg ttctgaaaac ggtgcggctt gcgaacgcgg 660  
 gataggagcc gggacgacac tccgtcgaca ggttcaacag cttgttgaca gagaacacga 720  
 tacaaattga gatgccttgg tttggaagtt gaaactgcag tgaagtggac ctagcttgcc 780  
 atccggtgcc tgctacaatt gctaaacaga ggcggcatcc gagcccagca gccacaggac 840  
 aagtggacgc ttgcagattg ttcgttcctt gaaatggaga aaactgtgat gttcctagaa 900  
 aaagtgaaag aaaacggtgc cctaaagacc atgaggtccg tcctaaagcg agacaccgtc 960  
 gagaagtgtc caaaatttca gcgcattttg ctacaggagg cttccggaga acccggaaaa 1020

aaggaaggga actccacact tcttgaaggc aatcggcgaa caagaagtga gggaagggcg 1080  
gcttttgaga agcaggggaac aacaacacag tctgagacga aaagtaagca caaaagggttc 1140  
accgtgaaaa ctcggttcgt ccgagaggcc gaagagattg tctacgccgg gcacagacaa 1200  
gcgtcttttt ctgcgacact cgacc 1225

<210> 285

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 285

gggcgagaac atcaccattg 20

<210> 286

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 286

acacgaagga cctgtatgg 19

<210> 287

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 287

gtgcttcgat ttgaatgcg 19

<210> 288

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 288

tcaaatcgaa gcacatg

17

<210> 289

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 289

tttcccagac cttgctgtc

19

<210> 290

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 290

tcattcaggt ccatcgtcgg

20

<210> 291

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

20

<213> *Toxoplasma gondii*

$\langle 222 \rangle$  (1) . . (222)

tcaagaagat cttcggcgag gcgaaggcag cgccacaggc cgccgccaag aaqqaqactc 662

ccaaagccgc ggcgaaacct gcacagagcg ccggcgatga cgaagaaccg gcgaagaagc 722  
 ctgcagtcaa gtgcgagttg gacttgctcc cagagccgac gatggacctg aatgagtgga 782  
 agcgcggtgta ctccaacacg aaggacctgt atggcacagc gatgaaatgg ttctgggaac 842  
 acctcgacgc ggcagggtat tccttgtggt acatgaaata tcagaaactc gagggcgagt 902  
 gcaccgtcgc gttcgtcacc tcgaaccagc tcggcggtt cctgcagcgg atcgacctgg 962  
 ccttcgcaa atactccttc ggcgtcgtcg acgtgatggg cgagaacggc tgcttcgaca 1022  
 tcgaggggtgt ctggctgttc cgcggccaag acgtaccag cttgatgaag gaccacctgt 1082  
 cgtacgagta ccacacttgg cagaaactcg acgtcgccag cgcaaaagac aagcaactcg 1142  
 tcgcagactt ttggtgcgcg tgcgacgaca tccaaggctc ccccatcgcc gacagcaagg 1202  
 tctggaaata aaaggaaata acgcacttcg cgaaacgaag gggcggaac agaggtgtgt 1262  
 gtctttggtg ctgtgaaaaa aagcgacgcg taaaaaacgg cgagaaatgt tcgtggcgtg 1322  
 gcgtgcggtg agaggggtac agtggcgaag ggtcacaac ccatgtgctt cgatttgaat 1382  
 gcgcttccat ctgtacacct ggctcttcg tgcgtccttt cagtctctcc taaaaatctc 1442  
 gtttcacgcg ggtgcagttg cggtacttca ggagcttcgc aggcgccgct cgcgcgcgct 1502  
 ccccgctcta ggaactctca cagacccca tttatgtcaa ctcgaaaaaa aaaaaaaaaa 1562  
 aaaaaaaaaa a 1573

&lt;210&gt; 293

&lt;211&gt; 73

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 293

Ala Ala Ser Leu Phe Thr Leu Arg Lys Asn Glu Thr Pro Asp Ala Gln  
 1 5 10 15

Gly Arg Cys Ala Arg Pro Glu Gly Ala Val Gly Gly Gly Phe Pro Gly  
 20 25 30

Pro Ala Ala Ala Asp Cys Ala Phe His Arg Gly Glu Gly Arg Gln Gly  
 35 40 45

Ser Gly Val Leu Gly Gln Val Ala Ser Gly Ala Ser Ala Pro Val Gly  
 50 55 60

Val Arg Gly Arg Arg Arg Val Ser Val  
 65 70

<210> 294

<211> 1573

<212> DNA

<213> *Toxoplasma gondii*

<400> 294

tttttttttt tttttttttt tttttttcga gttgacataa atgggggtcgt gtgagagttc 60  
 ctagagcgagg gagcgcgcg gagcgggcgcc tgcgaagctc ctgaagtacc gcaactgcac 120  
 ccgcgtgaaa cgagattttt aggagagact gaaaggacgc acggaagagc caggtgtaca 180  
 gatggaagcg cattcaaattc gaagcacatg ggtttgtgac ctttcgccac tgtaccctc 240  
 tcaccgcacg ccacgccacg aacattttct gccgtttttt acgcgtcgtt tttttcaca 300  
 gcaccaaaga cacacacctc tgttgccgccc ctttcgtttc gcgaagtgcg ttatttcctt 360  
 ttatttcag accttgctgt cggcgatggg gcgacctgg atgtcgtcgc acgcgcacca 420  
 aaagtctgcg acgagttgct tgtcttttgc gctggcgacg tcgagtttct gccaaagtgtg 480  
 gtactcgtac gacgggtggt cttcatcaa gctgggtacg tcttgccgc ggaacagcca 540  
 gacaccctcg atgtcgaagc agccgtttc gccatcacg tcgacgacgc cgaaggagta 600  
 tttgcggaag gccgggtcga tccgtgcag gaagccgccc agctggttcg aggtgacgaa 660  
 cgcgacggtg cactcgccct cgagttttct atatttcatt taccacaagg aataccctgc 720  
 cgcgctcagg tgttcccaga accatttcatt cgctgtgcca tacaggtcct tcgtgttgga 780  
 gtacacgcgc ttccattcat tcaggtccat cgctggctct gggagcaagt ccaactcgca 840  
 cttgactgca ggcttcttcg ccggtttctt gtcacgccc gcgctctgtg caggcttcgc 900  
 cgcggtcttg ggagtctcct tcttgccgga ggctgtggc gctgccttcg cctcgccgaa 960  
 gatcttcttg aagctcttct gattcaccac ggtctcgtag aagcgcttca agtacggacg 1020  
 cttctcgagc agcgctgcgg cgtccacctt gccggaccgg aagccgtagc tcagcaccgc 1080



gacgaggcac aagtctgcaa tggatgatgtt ctgcccacc atgaagggtgc gcgtcttcag 1140  
gtgggcgctcg accgcgttca gtgcctgcgc caggtcgctc tgcgcgcgct ccgcaacett 1200  
tccccctgc accaagcaac acatcggaat ctgacttcg agggtcgaga agtccaacca 1260  
catgtccact tgtccctgct ccgcaagcgt ctgcgcgtac aggcacttgt cggcgcgaag 1320  
tcgcgcgagg aagcggcaaa tcgcgttgct ttcaaacaga cacacgccgc cgacctcgga 1380  
ctccaacagg ggcagacgcc ccagaggcga cttggccaag aacgccgat ccttgctcgc 1440  
cttccccacg gtgaaaggca cagtctgcag cggcagggtcc aggaaagccg ccaccaactg 1500  
caccttccgg ccgcgcacat cgtccttggg cgtcaggagt ttcattttta cggaggggtga 1560  
agagagaggc tgc 1573

&lt;210&gt; 295

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 295

ttatttcccc gctcgtctc 20

&lt;210&gt; 296

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 296

cctactgtga ctccatcac 20

&lt;210&gt; 297

&lt;211&gt; 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 297

atcaccacta agcgtaggg

19

<210> 298

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 298

tcgaaagaac gaagctgcc

19

<210> 299

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 299

cgaagggtgt catcctctac

20

<210> 300

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 300

tatcgccaac agagtgaacc g

21

<210> 301

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 301

gtggacccaa gcatttgtgt gg

22

<210> 302

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 302

gcgtttcgac agttctatca c

21

<210> 303

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 303

acggaagaag acggagatgg

20

<210> 304

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

## Primer

&lt;400&gt; 304

gagattccct acgcttagtg

20

&lt;210&gt; 305

&lt;211&gt; 19

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 305

gttcgtcttt cgccataac

19

&lt;210&gt; 306

&lt;211&gt; 2417

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(27)

&lt;400&gt; 306

gtt tcc tcc gtt ctt ctt cgt ttt aaa ctgcaggaag tttctcctcg

47

Val Ser Ser Val Leu Leu Arg Phe Lys

1

5

ccttcgaccg cgctccgggga ctgaggtctg ctgtccacgc aggacttctt cttctccttt 107

ttcgcgccgc cagacgaggt cttcccgact ttcgtctgc acgcttctcc gcatctccgc 167

atctcccat ctccgtcttc ttccgttttt gactgtctgc gctctttctc cgacctcgct 227

cgaacgccgc gattctcgcc ttttatttcc ccgctctgtc tccggccggt ccacgcacct 287

tcctcggccg ttgcctcgcg ttctctgggt gtacagacac ctgagcgctc tgcgttgctg 347

cagcatttct ctgagccgca acatggggaa tgcgcagcct cgcggcggag gcttcccagg 407

aggctctgaa gaagacaaga agaaggagag aaagagactt gaagctgcgc ctccaacgca 467

cattggaaaa agaaagaaga aaggaaaagg ccccgctcgggt cacagcagac ttctactgt 527

gactcccatc accaagtgtc gcctgcgtct gctccgactc gagcgcataa aagactacct 587  
tcttctggag gaagagtata ttctcaacca ggagcagcgg aagccggcgg aggagaagaa 647  
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gcggaaggcg aaggagaagg cgctgtatca gaagaaaggg aacattccag agagtctgta 1547  
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agaggctttc cagagcgcag acgcgacaga tttgccgaag gaaaaaagtc acgaacgtgt 2207  
ctgtttgaga gaagaaccag ggccggcaca ggttctccgt gtatttttta ggaaggatcg 2267  
tgttgacagc gacaagaaag ccacacaaat gcttgggtcc acgggagcgc tgtccccag 2327  
acacacgcag aaatacgagt gaaacttact ctgcttcctg cgacaacttt cgcaattacg 2387  
agtggaacgc ttcaaaaaaa aaaaaaaaaa 2417

&lt;210&gt; 307

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 307

Val Ser Ser Val Leu Leu Arg Phe Lys

1

5

&lt;210&gt; 308

&lt;211&gt; 2417

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 308

ttttttttt ttttttgaa gcgttccact cgtaattgcg aaagttgtcg caggaagcag 60  
agtaagtttc actcgtattt ctgcgtgtgt ctgggggaca gcgctcccgt ggaccaagc 120  
atttgtgtgg ctttcttgtc gctgtcaaca cgatccttcc taaaaaatac acggagaacc 180  
tgtgccggcc ctggttcttc tctcaaacag acacgttcgt gacttttttc cttcggcaaa 240  
tctgtcgcgt ctgcgctctg gaaagcctct tcgctttcga ctgaattcgc tactcccact 300  
ccctacccg cggaatcgc cgagtgcgac tacgcagttc acatcttttt tcttgcagtc 360  
gccaagaaaa aaggtctgaa cggttcactc tgttggcgat aaacggtttc gctttgagtg 420

ccccactcc gcgatcatgc gtgtccacac tggcaactca actcgaaaaa atcagacacg 480  
cagacgctaa gttttcgggc aaacaacctt ttcttttgaa tctctcttgt cttcttgacg 540  
tgcgacctcg tcatttctat ttctgcagcg ttctgacagt tctatcacag agaaaccgaa 600  
gccacagggg gtgcgctacc ggcggcgcct caaaactgac gagtcgaaag cgctcgacac 660  
gcctttcttc tcagcagaag atgtctctga agcctgctgt gacattttcc agtctcttct 720  
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tcgttcgcca cggccttcgc gagcagagtc ttctctgtcc cgggggggtc gtagaggatg 1380  
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tcaagcgggtg ccttctccac ttcatgacc gagatgagag ggtccacctc gtcggtcaaa 1560  
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agacgcaggc gacacttggg gatgggagtc acagtaggaa gtctgctgtg accgacgggg 1920  
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 ctctccttct tcttgtcttc ttcagagcct cctgggaagc ctccgccgag aggtgcgca 2040  
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 aaaaacggaa gaagacggag atggggagat gcggagatgc ggagaagcgt gcagacgaaa 2280  
 agtcgggaag acctcgtctg gcggcgcgaa aaaggagaag aggaagtcct gcgtggacag 2340  
 cagacctcag tccccggacg cggtcgaagg cgaggagaaa cttctgcag tttaaaacga 2400  
 agaagaacgg aggaaac 2417

<210> 309

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 309

cggactgcgt tatcgttacc

20

<210> 310

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 310

ctgttgcctt cgatatg

18

<210> 311



&lt;211&gt; 1785

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(75)

&lt;400&gt; 311

gaa ccg gtg cac ttt tgc acg tgt acg caa gac agc ttc gca gac aac 48  
 Glu Pro Val His Phe Cys Thr Cys Thr Gln Asp Ser Phe Ala Asp Asn  
     1                    5                    10                    15

atc tgg cag cct ccc gct cat ttt tag tcagcaaaaa tggcacccgc 95  
 Ile Trp Gln Pro Pro Ala His Phe  
                     20                    25

acttggtgcag aggagaaaaga aggtggccat gattggctct ggcatgattg gtgacactat 155  
 gggctacctg tgcgctctcc gtgagctcgc tgacgtcgtt ctctacgatg ttctcaaaag 215  
 tatgcccgag ggtaaggctc ttgacctgag ccatgtgacc tccgtggctg aacccaacgt 275  
 ttccgtccgt gctgagtact cttacgaggc cgcgctcacc ggtgcggact gcattatcgt 335  
 taccgcgggt ctgaccaagg tgccggggcaa gcccgactcc gagtggagcc gaaacgatct 395  
 gctcccgttc aactogaaga tcattcgca gatcggtcag aacatcaaqa aatctgccc 455  
 caagaccttc atcatcgtgg tcaccaaccc gctggactgc atggtcaagg tcatgtgca 515  
 ggctcttggc gtcccgacca acatgatctg cggatatggc tgcattgctg actctatctg 575  
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 catcggcaca caccggcact gcatggctcc gcttgctcgg tacattaccg taaatctcta 695  
 cccgatccag aagttcatca aggaacggct agtcacggag aagcagctcg aatctatcga 755  
 tgagcacacc aaagtgtctg gcggcgagat cgtccgcttc ctccggccagg gtctcctct 815  
 ctacgcccc gccgcatccg ctgtcgccat ggcaacatcc ttcttgaacg acgaaagcga 875  
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 tctcccggcc gtcattggag gcgcggcat cgagcgcgtc atcgagctcg aactgaacga 995  
 ggaggagaag aagcagttcc agaagtcctg cgacgacgtc atggcgctca acaagccggt 1055

tgctgctctt caggcgtaag cgttggcaaa acaggagcgg aatgccactt tactgcgcgg 1115  
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 cgcgctcgtc ccgaaaaagg gaagtcgcgg cgctgtcggg caacgctgtg cgggttgag 1235  
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 acaggtgaac gcgtgcaagc atgagaggca tccgtcgtg cgttcgctca catatccgaa 1355  
 ggcaacagat tttggtcggc aaaagcctct gcacaaccgt ggcaagatga tggaaacagt 1415  
 tcgtgtttgg acagcaaccg cgttcgctct cactcaaaac cctgtagtcg agaggggtgt 1475  
 cgcgtgactt ggcttttgtg ggagtgccca aatcgctctgt gttcgagggtg aagatcacat 1535  
 gtccgtgca gtactgaaaa acacttggtg cgcagaggcg agcgataggt gcgctgactt 1595  
 tttttttgtt tgttcaagga aggcattctt tttttttta ccggttgctc actgtcatgt 1655  
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 tctcatggag catttttcaa cgttgcttca aacgaatgct ggtttcaaac aaaaaaaaaa 1775  
 aaaaaaaaaa 1785

&lt;210&gt; 312

&lt;211&gt; 24

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 312

Glu Pro Val His Phe Cys Thr Cys Thr Gln Asp Ser Phe Ala Asp Asn  
 1 5 10 15

Ile Trp Gln Pro Pro Ala His Phe  
 20

&lt;210&gt; 313

&lt;211&gt; 1785

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 313

gaaccggtgc acttttgcac gtgtacgcaa gacagcttcg cagacaacat ctggcagcct 60

cccgcctcatt tttagtcagc aaaaatggca cccgcacttg tgcagaggag aaagaaggtg 120  
gccatgattg gctctggcat gattggtggc actatgggct acctgtgcgc tctccgtgag 180  
ctcgcctgacg tcgttctcta cgatgttgtc aaaggtatgc ccgagggtaa ggctcttgac 240  
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gaggccgcgc tcaccggtgc ggactgcgtt atcgttaccg ccggtctgac caaggtgccg 360  
ggcaagcccg actccgagtg gagccgaaac gatctgctcc cgttcaactc gaagatcatt 420  
cgcgagatcg gtcagaacat caagaagtac tgccccaaga cttcatcat cgtggtcacc 480  
aaccgcctgg actgcatggt caaggtcatg tgcgaggcct ctggcgctcc gaccaacatg 540  
atctgcggta tggcctgcat gctcgactct ggtcgcttcc gccgatacgt cgcgcacgcg 600  
ctgtctgtct ctccccgcga cgtccaggcc accgtcatcg gcacacacgg cgaactgcatg 660  
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gccccaaatcg tctgtgttcg aggtgaagat cacatgtccg ctgcagtact gaaaaacact 1560  
tggtgcgcag aggcgagcga taggtgcgct gacttttttt ttgtttgttc aaggaaggca 1620  
tctttttttt ttttaccggt tgtccactgt catgtcgaaa acgtagtccg tgtgaagtgg 1680  
ttgggtccct gttgtccttt gtctagccgc gcgtgtctca tggagcattt ttcaacgttg 1740  
cttcaaacga atgctgggtt caaacaaaaa aaaaaaaaaa aaaaa 1785

<210> 314

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 314

tctccgactg tggagtgc

18

<210> 315

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 315

gatggcatgg atttgacc

18

<210> 316

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 316

tgaggagacc gaaaagg

17

<210> 317

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 317

tcagtgtctt gacgaatcc

19

<210> 318

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 318

gccttcagga gagaagctac

20

<210> 319

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 319

gatccaagat gacgcatggg

20

<210> 320

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 320

gaaactctgg ggaaaacggc

20

<210> 321

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 321

cttcttcgct ctcaaacatc

20

<210> 322

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 322

atcacggttt gtcgcac

17

<210> 323

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 323

cggacttcct tatgatcgg

19

<210> 324

<211> 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 324

agtcggagaa ggcaccatag

20

&lt;210&gt; 325

&lt;211&gt; 17

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 325

ttctctctcc ttttcgg

17

&lt;210&gt; 326

&lt;211&gt; 2167

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1716)

&lt;400&gt; 326

gtg	agc	acc	cgc	cgg	aga	gaa	gac	acc	gag	agc	gag	gcg	tgc	gtc	aac	48
Val	Ser	Thr	Arg	Arg	Arg	Glu	Asp	Thr	Glu	Ser	Glu	Ala	Cys	Val	Asn	
1				5					10					15		

caa	gtc	gaa	acc	atg	gag	tgc	cca	act	cag	gct	ggg	caa	gca	tgc	ggc	96
Gln	Val	Glu	Thr	Met	Glu	Cys	Pro	Thr	Gln	Ala	Gly	Gln	Ala	Cys	Gly	
			20						25					30		

aac	tat	ggt	gcc	ttc	tcc	gac	tgt	gga	gtg	cct	ctt	cgc	ggc	ttc	gcc	144
Asn	Tyr	Gly	Ala	Phe	Ser	Asp	Cys	Gly	Val	Pro	Leu	Arg	Gly	Phe	Ala	
		35					40					45				

atg	gcc	ttc	ccc	gag	aac	tgc	cca	gag	ctc	gtg	gcc	ttc	gcc	gcc	tgc	192
Met	Ala	Phe	Pro	Glu	Asn	Cys	Pro	Glu	Leu	Val	Ala	Phe	Ala	Ala	Cys	

50	55	60	
gat gct ccc gcg cct ccc caa gag gac cgc tgc cat tct ttc tcg gcc			240
Asp Ala Pro Ala Pro Pro Gln Glu Asp Arg Cys His Ser Phe Ser Ala			
65	70	75	80
tgg tcc aag tgc aca cac ata ccc ggc act act ctg tac gag cag acg			288
Trp Ser Lys Cys Thr His Ile Pro Gly Thr Thr Leu Tyr Glu Gln Thr			
	85	90	95
cgc tcc tgc gat ggc atg gat ttg acc gag tcc cgc ttc tgc act ccc			336
Arg Ser Cys Asp Gly Met Asp Leu Thr Glu Ser Arg Phe Cys Thr Pro			
	100	105	110
gac gag gag gtc ggc tcg gac gtt tcc act gac gtc gct tcc gaa tgc			384
Asp Glu Glu Val Gly Ser Asp Val Ser Thr Asp Val Ala Ser Glu Cys			
	115	120	125
ggt tcc ctc ggc gag ttc ggc gag tgt gtg aac ggc ctt cag gag aga			432
Gly Ser Leu Gly Glu Phe Gly Glu Cys Val Asn Gly Leu Gln Glu Arg			
	130	135	140
agc tac tcg gac tgc ccc gat cat aag gaa gtc cgt cag tgc tct gac			480
Ser Tyr Ser Asp Cys Pro Asp His Lys Glu Val Arg Gln Cys Ser Asp			
145	150	155	160
gaa tcc tgc tct gcc ttc ggc gag tgg tca ccc tgc ggg gaa ccc cag			528
Glu Ser Cys Ser Ala Phe Gly Glu Trp Ser Pro Cys Gly Glu Pro Gln			
	165	170	175
caa ggc ctg cgt atc cgc aag aga cgt gca tgc gac aac gtg cac tgc			576
Gln Gly Leu Arg Ile Arg Lys Arg Arg Ala Cys Asp Asn Val His Cys			
	180	185	190
gcc tgt gtc gag gcc gag gtc tgc ggc gat gtc acc cca gag att gag			624
Ala Cys Val Glu Ala Glu Val Cys Gly Asp Val Thr Pro Glu Ile Glu			
	195	200	205
gag gaa gaa ggc gaa cat ttc ccc cct gaa gaa ggc gag gtc ttg cct			672
Glu Glu Glu Gly Glu His Phe Pro Pro Glu Glu Gly Glu Val Leu Pro			
	210	215	220
cca tat gaa gag ggt cct ggt gag ggt gag ctt gtt cct ccc gag gag			720
Pro Tyr Glu Glu Gly Pro Gly Glu Gly Glu Leu Val Pro Pro Glu Glu			
225	230	235	240
gag atc cct gaa gga gaa cat gtt cct gag gag gaa atc cct gaa gga			768
Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly			



	245	250	255	
gaa cat att cct gaa gag ctc cca gaa ggc gag cat gtt cct gag gag				816
Glu His Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu				
	260	265	270	
gaa atc cct gaa gga gaa cat gtt cct gaa gag gaa atc cct gaa gga				864
Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly				
	275	280	285	
gag cat gtt cct gaa gag ttc cca gaa ggc gag cat gtt cct gag gag				912
Glu His Val Pro Glu Glu Phe Pro Glu Gly Glu His Val Pro Glu Glu				
	290	295	300	
gaa atc cct gaa gga gaa cat gtt cct gaa gag gaa atc cct gaa gga				960
Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly				
	305	310	315	320
gag cat gtt cct gaa gag ttc cct gaa gga gag cat att cct gag gag				1008
Glu His Val Pro Glu Glu Phe Pro Glu Gly Glu His Ile Pro Glu Glu				
	325	330	335	
ctc cct gaa ggc gag cat atc cct gaa gag ttc cct gaa gga gag cat				1056
Leu Pro Glu Gly Glu His Ile Pro Glu Glu Phe Pro Glu Gly Glu His				
	340	345	350	
att cct gag gag ctc cct gaa ggc gag cat gtt cct gag gag gag atc				1104
Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile				
	355	360	365	
cct gaa gga gag cat att cct gaa gag ttc cca gaa ggc gag cat gtt				1152
Pro Glu Gly Glu His Ile Pro Glu Glu Phe Pro Glu Gly Glu His Val				
	370	375	380	
cct gag gag gaa atc cct gaa gga gaa cat att cct gag gag gag ttc				1200
Pro Glu Glu Glu Ile Pro Glu Gly Glu His Ile Pro Glu Glu Glu Phe				
	385	390	395	400
cct gaa gga gag cat gtt cct gag gag gag atc cct gaa ggc gag cat				1248
Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His				
	405	410	415	
gtt cct gag gag gag ctc cct gga gga gaa ctt att cct gag gag gag				1296
Val Pro Glu Glu Glu Leu Pro Gly Gly Glu Leu Ile Pro Glu Glu Glu				
	420	425	430	
atc cct gaa gga gag cat gtt cct gaa gag ctc cct gaa ggc gag cat				1344
Ile Pro Glu Gly Glu His Val Pro Glu Glu Leu Pro Glu Gly Glu His				

435	440	445	
gtt cct gag gag gag atc cct gaa gga gag cat gtt cct gaa gag gaa			1392
Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu			
450	455	460	
atc cct gaa ggc gag cat gtt cct gag gag gag acc cct gaa gga gaa			1440
Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Thr Pro Glu Gly Glu			
465	470	475	480
cat gct cca gag gaa gag act cct gca cct gag gag acc gaa aag gag			1488
His Ala Pro Glu Glu Glu Thr Pro Ala Pro Glu Glu Thr Glu Lys Glu			
485	490	495	
gag gaa gaa ggc gtg cca gtc gca gcg att gcc ggt ggt gtc gtc gga			1536
Glu Glu Glu Gly Val Pro Val Ala Ala Ile Ala Gly Gly Val Val Gly			
500	505	510	
ggt gtg ttg ctc att gct ggt ggt gca ggt gct gcc gtg tac gca aac			1584
Gly Val Leu Leu Ile Ala Gly Gly Ala Gly Ala Ala Val Tyr Ala Asn			
515	520	525	
caa ggt ggc gtt gaa gca gct gaa gac gaa gtg atg ttt gag agc gaa			1632
Gln Gly Gly Val Glu Ala Ala Glu Asp Glu Val Met Phe Glu Ser Glu			
530	535	540	
gaa gac gga acc cag gct ggc gag aac cgc gag agc gag acg gtc att			1680
Glu Asp Gly Thr Gln Ala Gly Glu Asn Arg Glu Ser Glu Thr Val Ile			
545	550	555	560
gag atc gaa gat gac gca tgg gca gac atg gac taa agtagactag			1726
Glu Ile Glu Asp Asp Ala Trp Ala Asp Met Asp			
565	570		
tagtctgtgt gggcacatgc aggcgtgcga caaaaccgtg atcgcgaggt attctgtgtt			1786
acggggcgag cgtctgcggc tgctccttcga aggggaggcg gcgtgacact ctgagctagg			1846
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gtgttaatgc gataaataac taatgaagca aggttatgaa cagcttcgtg agctatatcc			2146

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2167

&lt;210&gt; 327

&lt;211&gt; 571

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 327

Val Ser Thr Arg Arg Arg Glu Asp Thr Glu Ser Glu Ala Cys Val Asn  
 1 5 10 15

Gln Val Glu Thr Met Glu Cys Pro Thr Gln Ala Gly Gln Ala Cys Gly  
 20 25 30

Asn Tyr Gly Ala Phe Ser Asp Cys Gly Val Pro Leu Arg Gly Phe Ala  
 35 40 45

Met Ala Phe Pro Glu Asn Cys Pro Glu Leu Val Ala Phe Ala Ala Cys  
 50 55 60

Asp Ala Pro Ala Pro Pro Gln Glu Asp Arg Cys His Ser Phe Ser Ala  
 65 70 75 80

Trp Ser Lys Cys Thr His Ile Pro Gly Thr Thr Leu Tyr Glu Gln Thr  
 85 90 95

Arg Ser Cys Asp Gly Met Asp Leu Thr Glu Ser Arg Phe Cys Thr Pro  
 100 105 110

Asp Glu Glu Val Gly Ser Asp Val Ser Thr Asp Val Ala Ser Glu Cys  
 115 120 125

Gly Ser Leu Gly Glu Phe Gly Glu Cys Val Asn Gly Leu Gln Glu Arg  
 130 135 140

Ser Tyr Ser Asp Cys Pro Asp His Lys Glu Val Arg Gln Cys Ser Asp  
 145 150 155 160

Glu Ser Cys Ser Ala Phe Gly Glu Trp Ser Pro Cys Gly Glu Pro Gln  
 165 170 175

Gln Gly Leu Arg Ile Arg Lys Arg Arg Ala Cys Asp Asn Val His Cys  
 180 185 190

Ala Cys Val Glu Ala Glu Val Cys Gly Asp Val Thr Pro Glu Ile Glu  
 195 200 205

Glu Glu Glu Gly Glu His Phe Pro Pro Glu Glu Gly Glu Val Leu Pro  
 210 215 220  
 Pro Tyr Glu Glu Gly Pro Gly Glu Gly Glu Leu Val Pro Pro Glu Glu  
 225 230 235 240  
 Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly  
 245 250 255  
 Glu His Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu  
 260 265 270  
 Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly  
 275 280 285  
 Glu His Val Pro Glu Glu Phe Pro Glu Gly Glu His Val Pro Glu Glu  
 290 295 300  
 Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly  
 305 310 315 320  
 Glu His Val Pro Glu Glu Phe Pro Glu Gly Glu His Ile Pro Glu Glu  
 325 330 335  
 Leu Pro Glu Gly Glu His Ile Pro Glu Glu Phe Pro Glu Gly Glu His  
 340 345 350  
 Ile Pro Glu Glu Leu Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile  
 355 360 365  
 Pro Glu Gly Glu His Ile Pro Glu Glu Phe Pro Glu Gly Glu His Val  
 370 375 380  
 Pro Glu Glu Glu Ile Pro Glu Gly Glu His Ile Pro Glu Glu Glu Phe  
 385 390 395 400  
 Pro Glu Gly Glu His Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His  
 405 410 415  
 Val Pro Glu Glu Glu Leu Pro Gly Gly Glu Leu Ile Pro Glu Glu Glu  
 420 425 430  
 Ile Pro Glu Gly Glu His Val Pro Glu Glu Leu Pro Glu Gly Glu His  
 435 440 445  
 Val Pro Glu Glu Glu Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu  
 450 455 460

Ile Pro Glu Gly Glu His Val Pro Glu Glu Glu Thr Pro Glu Gly Glu  
465 470 475 480

His Ala Pro Glu Glu Glu Thr Pro Ala Pro Glu Glu Thr Glu Lys Glu  
485 490 495

Glu Glu Glu Gly Val Pro Val Ala Ala Ile Ala Gly Gly Val Val Gly  
500 505 510

Gly Val Leu Leu Ile Ala Gly Gly Ala Gly Ala Ala Val Tyr Ala Asn  
515 520 525

Gln Gly Gly Val Glu Ala Ala Glu Asp Glu Val Met Phe Glu Ser Glu  
530 535 540

Glu Asp Gly Thr Cln Ala Gly Glu Asn Arg Glu Ser Glu Thr Val Ile  
545 550 555 560

Glu Ile Glu Asp Asp Ala Trp Ala Asp Met Asp  
565 570

<210> 328

<211> 2167

<212> DNA

<213> Toxoplasma gondii

<400> 328

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ggaaaacagg aaactctggg gaaaacggct ttccacaagg aggccgcagt agttttatt 180  
tctacagcgg cttgtgacac acgaagctgc ggcagcctat atataagctg ctggaagaa 240  
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aatggctgcg ttcgtctggt acctagctca gagtgtcacg ccgcctcccc ttctaaagaa 360  
agccgcagac gctccgcccg taacacagaa tacctcgcga tcacggtttt gtcgaagaa 420  
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cgatctcaat gaccgtctcg ctctcgcggt tctcgcacgc ctgggttccg ttttttttt 540  
tctcaaakat cacttcgtct tcagctgctt caacgccacc ttgggtttgcg tacacggaag 600

cacctgcacc accagcaatg agcaacacac ctccgacgac accaccggca atcgctgcga 660  
ctggcacgcc ttcttctctc tccttttcgg tctctcagg tgcaggagtc tcttctcttg 720  
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tgaccaggc cgagaaagaa tggcagcggc cctcttgggg aggcgcggga gcacgcagg 1980  
cggcgaaggc cagcagctct gggcagttct cggggaaggc catggcgaag ccgcgaagag 2040

gcactccaca gtcggagaag gcaccatagt tgccgcatgc ttgccagcc tgagttgggc 2100

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tgctcac 2167

<210> 329

<211> 2393

<212> DNA

<213> *Toxoplasma gondii*

<220>

<221> CDS

<222> (1)..(594)

<400> 329

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Ala Gly Ile Leu Ala Thr Thr Gly Val His Ser Leu Ala Ala Ile Pro  
1 5 10 15

aga ctc acc tca gca gcc gct act gtg tgg acc tcc cag aca agg ttg 96  
Arg Leu Thr Ser Ala Ala Ala Thr Val Trp Thr Ser Gln Thr Arg Leu  
20 25 30

agt cga tca cct gcg aag tcg gct ccc tgc ccc agc ccc cga ctg tcg 144  
Ser Arg Ser Pro Ala Lys Ser Ala Pro Cys Pro Ser Pro Arg Leu Ser  
35 40 45

ccc ccg gcg agg gtg gtg agg aag acg gca acg cat gtg gac cgt ggg 192  
Pro Pro Ala Arg Val Val Arg Lys Thr Ala Thr His Val Asp Arg Gly  
50 55 60

tcc gtg gag ccc gtg tcc cgg cga agg aaa caa cat gag cac ccg ccg 240  
Ser Val Glu Pro Val Ser Arg Arg Arg Lys Gln His Glu His Pro Pro  
65 70 75 80

gag aga aga cac cga gag cga ggc gtg cgt caa cca agt cga aac cat 288  
Glu Arg Arg His Arg Glu Arg Gly Val Arg Gln Pro Ser Arg Asn His  
85 90 95

gga gtg ccc aac tca ggc tgg gca agc atg cgg caa cta tgg tgc ctt 336  
Gly Val Pro Asn Ser Gly Trp Ala Ser Met Arg Gln Leu Trp Cys Leu  
100 105 110

ctc cga ctg tgg aat gcc tct tcg cgg ctt cgc cat ggc ctt ccc cga 384  
Leu Arg Leu Trp Asn Ala Ser Ser Arg Leu Arg His Gly Leu Pro Arg  
115 120 125

gaa ctg ccc aga gct cgt ggc ctt cgc cgc ctg cga tgc tcc cgc gcc 432  
 Glu Leu Pro Arg Ala Arg Gly Leu Arg Arg Leu Arg Cys Ser Arg Ala  
 130 135 140

tcc cca aga gga ccg ctg cca ttc ttt ctc ggc ctg gtc caa gtc cac 480  
 Ser Pro Arg Gly Pro Leu Pro Phe Phe Leu Gly Leu Val Gln Val His  
 145 150 155 160

aca cat acc cgg cac tac tct gta cga gca gac gcg ctc ctg cga tgg 528  
 Thr His Thr Arg His Tyr Ser Val Arg Ala Asp Ala Leu Leu Arg Trp  
 165 170 175

cat gga ttt gac cga gtc ccg ctt ctg cac tcc cga cga gga ggt cgg 576  
 His Gly Phe Asp Arg Val Pro Leu Leu His Ser Arg Arg Gly Gly Arg  
 180 185 190

ctc gga cgt ttc cac tga cgtcgtctcc gaatgcgggtt ccctcggcga 624  
 Leu Gly Arg Phe His  
 195

gttcggcgag tgtgtgaacg gccttcagga gagaagctac tcggactgcc ccgacataa 684

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tgagcttggt cctcccgagg aggagatccc tgaaggagaa catgttctg aggaagaaat 984

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acatatctct gaggaggagt ttctgaagg agagcatgtt cctgaggagg agatccctga 1464



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&lt;210&gt; 330

&lt;211&gt; 197

&lt;212&gt; PRT

<213> *Toxoplasma gondii*

&lt;400&gt; 330

Ala	Gly	Ile	Leu	Ala	Thr	Thr	Gly	Val	His	Ser	Leu	Ala	Ala	Ile	Pro
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Arg	Leu	Thr	Ser	Ala	Ala	Ala	Thr	Val	Trp	Thr	Ser	Gln	Thr	Arg	Leu
			20					25					30		

Ser	Arg	Ser	Pro	Ala	Lys	Ser	Ala	Pro	Cys	Pro	Ser	Pro	Arg	Leu	Ser
			35				40					45			

Pro Pro Ala Arg Val Val Arg Lys Thr Ala Thr His Val Asp Arg Gly  
 50 55 60  
 Ser Val Glu Pro Val Ser Arg Arg Arg Lys Gln His Glu His Pro Pro  
 65 70 75 80  
 Glu Arg Arg His Arg Glu Arg Gly Val Arg Gln Pro Ser Arg Asn His  
 85 90 95  
 Gly Val Pro Asn Ser Gly Trp Ala Ser Met Arg Gln Leu Trp Cys Leu  
 100 105 110  
 Leu Arg Leu Trp Asn Ala Ser Ser Arg Leu Arg His Gly Leu Pro Arg  
 115 120 125  
 Glu Leu Pro Arg Ala Arg Gly Leu Arg Arg Leu Arg Cys Ser Arg Ala  
 130 135 140  
 Ser Pro Arg Gly Pro Leu Pro Phe Phe Leu Gly Leu Val Gln Val His  
 145 150 155 160  
 Thr His Thr Arg His Tyr Ser Val Arg Ala Asp Ala Leu Leu Arg Trp  
 165 170 175  
 His Gly Phe Asp Arg Val Pro Leu Leu His Ser Arg Arg Gly Gly Arg  
 180 185 190  
 Leu Gly Arg Phe His  
 195

&lt;210&gt; 331

&lt;211&gt; 2392

&lt;212&gt; DNA

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 331

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 tctacagcgg cttgtgacac acgaagctgc ggcagcctat atataagctg ctggaaaaga 240  
 tacaccagtg gaaaacgtaa aataaaatgg cacgtgatca agaaagagcg gacggacaca 300

aatggctgcg ttcgctctggt acctagctca gagtgtcacg ccgcctcccc ttgaaggac 360  
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tgcatgtgcc cacacagact actagtctac tttagtccat gtctgcccat gcgtcatctt 480  
cgatctcaat gaccgtctcg ctctcgcggt tctcgccagc ctgggttccg tcttcttcgc 540  
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caggaaatg ttgccttct gggaactctt caggaaatg ctctcttca gggatttctt 1320  
cttcaggaa atgttctct tcagggatt cctctcagg aacatgctcg ccttctggga 1380  
gctcttcagg aatatgttct ccttcaggga tttctctc aggaacatgt tctcttcag 1440  
ggatctctc ctccgggagga acaagctcac cctcaccagg acctcttca tatggaggca 1500  
agacctgcc ttcttcagggt gggaaatgtt cgccttctc ctctcaatc tctggggtga 1560  
catgccgca gacctggcc tcgacacagg cgcagtgcac gttgtcgcat gcacgtctct 1620  
tgcgatagc caggccttgc tggggttccc cgcagggtga ccaactgccg aaggcagagc 1680  
aggattctc agagcactga cggacttctt targatcggg gcagtccag tagcttctt 1740

cctgaaggcc gttcacacac tcgccgaact cgccgagggg accgcattcg gaagcgacgt 1800  
cagtggaaac gtccgagccg acctcctcgt cgggagtgcg gaagcgggac tcggtcaaatt 1860  
ccatgccatc gcaggagcgc gtctgctcgt acagagtagt gccgggtatg tgtgtgcact 1920  
tggaccaggc cgagaaagaa tggcagcggg cctcttgggg aggcgcggga gcatcgacgg 1980  
cggcgaaggc cagcagctct gggcagttct cggggaaggc catggcgaag ccgcgaagag 2040  
gcattccaca gtcggagaag gcaccatagt tgccgcatgc ttgccagcc tgagttgggc 2100  
actccatggt ttcgacttgg ttgacgcacg cctcgtcttc ggtgtcttct ctccggcggg 2160  
tgctcatgtt gtttccttcg ccgggacacg ggctccacgg acccacggtc cacatgcgtt 2220  
gccgtcttcc tcaccacct cgccgggggc gacagtcggg ggctggggca gggagccgac 2280  
ttcgcaggtg atcgactcaa ccttgtctgg gaggtccaca cagtagcggc tgctgaggtg 2340  
agtctcggga tcgcagcgag tgaatgcact ccagtgggtg caagaattcc tg 2392

<210> 332

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 332

ggaactgcat ccgttcatga g

21

<210> 333

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 333

tcttaaagcg ttcgtggtc

19

<210> 334

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 334

ggcgaccaat ctgcgaatac acc

23

<210> 335

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 335

gcaccccttg agacagagct tgag

24

<210> 336

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 336

gggttctctt ctcgctcatc ttcc

24

<210> 337

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 337

agtcagaagc agtcaaggc

19

&lt;210&gt; 338

&lt;211&gt; 647

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 338

gaccccttag ccacttagac cgattcccca gacttgctcg gagatagtgt cagtgtcact 60  
actacacagc tcaaagaagc cacatcccggt gaaaaatttt atcgtctaca agcgtggggc 120  
ggttctttgt tcaactgttca cgtcacgtgt ggacatgccg ttgtgtcgtg gcagcaaata 180  
cgaagaagcc aaagacatcc gaaaccgccc gttcagagtc ggggagactg cctgggtttt 240  
cccacgagct gcatgttcca gctagatgca agcacctgca gtggggatgt atctccgaaa 300  
aggcgagcaa ttttgtccaa aaagggtgag ctaatcgtga aatgtccact gacatgcagc 360  
gtccgcttct gtctcagtag cgatcttcac ttccgctgct acccgcttc gtttcgtttc 420  
ccccattcc agatatccct gccgctgtgg cgcctggaag cgctcctcga ctgcattgag 480  
cattccgccc tcacaagact tttttttccc ttttgccaac gtcgagaacc tctcacgggc 540  
gagcaaagtc tagtggttgg tttcagtagc gcggtcgtg gctctgtgta tgactgacct 600  
gaagaagcaa agacttcttg caacgtagaa acgcaaaggc gcttctt 647

&lt;210&gt; 339

&lt;211&gt; 647

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 339

aagaagcgc tttgcgtctc tacgttgcaa gaagtcttg cttcttcagg tcagtcatac 60  
acagagccag cgaccgccgt actgaaacca aacactagac ttgctcgcc cgtgagaggt 120  
tctcgacgtt ggcaaaaggg aaaaaaagt cttgtgacgg cggaatgctc aatgcagtcg 180  
aggagcgctt ccaggcgcca cagcggcagg gatatctgga atggggggaa acgaaacgaa 240

cgcgggtagc acgcgaagtg aagatcggta ctgagacaga agcggacgct gcatgtcagt 300  
 ggacatttca cgattagctc accctttttg gacaaaattg ctgcctttt cggagataca 360  
 tccccactgc aggtgcttgc atctagctgg aacatgcagc tcgtgggaaa acccaggcag 420  
 tctccccgac tctgaacggg cggtttcgga tgtctttggc ttcttcgtat ttgctgccac 480  
 gacacaacgg catgtccaca cgtgacgtga acagtgaaca aagaaccggc ccacgcttgt 540  
 agacgataaa atttttcacg ggatgtggct tctttgagct gtgtagtagt gacactgaca 600  
 ctatctccga gcaagtctgg ggaatcggtc taagtggcta aaggatc 647

&lt;210&gt; 340

&lt;211&gt; 867

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(867)

&lt;400&gt; 340

atg gca gga agg cag gcg gcg ttg ttt ttg gtg gtg ctg tct gtg gcg 48  
 Met Ala Gly Arg Gln Ala Ala Leu Phe Leu Val Val Leu Ser Val Ala  
 1 5 10 15

gcg ggc cct gtc tcc cag ctt gct cgg gcg agc gac gac agc gtc gac 96  
 Ala Gly Pro Val Ser Gln Leu Ala Arg Ala Ser Asp Asp Ser Val Asp  
 20 25 30

agc gtc gaa acc gcg cgt cag cac atg gag ctg gct atc gag gct gac 144  
 Ser Val Glu Thr Ala Arg Gln His Met Glu Leu Ala Ile Glu Ala Asp  
 35 40 45

gaa gag atg cac gag gcc tac gac cct ttg ttg gaa ttc gtt gag acg 192  
 Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr  
 50 55 60

ttt cgg gaa atc aaa aaa gct gtt gag gaa gat gcg gct ctg agt aca 240  
 Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr  
 65 70 75 80

gat gcg atc gac cgc gtg tcc cag ttc gat ctg gtt tcc ctc cta gat 288  
 Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp  
 85 90 95

gtc atc cga gag gct gca caa gca aag ttc gat ctc ctc gga cgc ctc 336  
 Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu  
 100 105 110

att aca gac atc gcc agc gga atc ggc gag ggt gcc atg gct ctg atg 384  
 Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met  
 115 120 125

gga gag gag gct gcg ttc att agg cca agg agg tca aag aga ggg aaa 432  
 Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys  
 130 135 140

aag act aca act aca acc agt tca tcc aca agt acg agt aca acg acc 480  
 Lys Thr Thr Thr Thr Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Thr  
 145 150 155 160

acg aca tca act acc act act acc act acc acc act acg act act act 528  
 Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 165 170 175

aca act acg aca cca aca aca act aca aca acc aca aca act aca cca 576  
 Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro  
 180 185 190

aca aca acg aca aca acc aca aca act aca cca aca aca acg aca aca 624  
 Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr  
 195 200 205

acc aca aca act aca cca aca aca acg aca aca acc aca acg cca act 672  
 Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr  
 210 215 220

aca acg aca tct acg aca acc act acg act acc aca act act act aca 720  
 Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 225 230 235 240

cca act aca aca acg aca acc acg gaa cca aca act aca aca aca acc 768  
 Pro Thr Thr Thr Thr Thr Thr Thr Glu Pro Thr Thr Thr Thr Thr Thr Thr  
 245 250 255

acg gaa cca acc aca act aca agc aca acg acg act acg aca act aca 816  
 Thr Glu Pro Thr Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 260 265 270

acg act acg aca cca tct acg acg aca tcc acc acc act acc ctc gat 864  
 Thr Thr Thr Thr Pro Ser Thr Thr Thr Ser Thr Thr Thr Thr Thr Leu Asp  
 275 280 285



tag

867

&lt;210&gt; 341

&lt;211&gt; 288

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 341

Met Ala Gly Arg Gln Ala Ala Leu Phe Leu Val Val Leu Ser Val Ala  
 1 5 10 15

Ala Gly Pro Val Ser Gln Leu Ala Arg Ala Ser Asp Asp Ser Val Asp  
 20 25 30

Ser Val Glu Thr Ala Arg Gln His Met Glu Leu Ala Ile Glu Ala Asp  
 35 40 45

Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr  
 50 55 60

Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr  
 65 70 75 80

Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp  
 85 90 95

Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu  
 100 105 110

Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met  
 115 120 125

Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys  
 130 135 140

Lys Thr Thr Thr Thr Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Thr  
 145 150 155 160

Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 165 170 175

Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro  
 180 185 190

Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr

195                      200                      205  
 Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr  
 210                      215                      220  
 Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 225                      230                      235                      240  
 Pro Thr Thr Thr Thr Thr Thr Thr Thr Glu Pro Thr Thr Thr Thr Thr Thr Thr Thr  
 245                      250                      255  
 Thr Glu Pro Thr Thr Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 260                      265                      270  
 Thr Thr Thr Thr Pro Ser Thr Thr Thr Ser Thr Thr Thr Thr Thr Leu Asp  
 275                      280                      285

&lt;210&gt; 342

&lt;211&gt; 867

&lt;212&gt; DNA

<213> *Toxoplasma gondii*

&lt;400&gt; 342

atggcaggaa ggcaggcggc gttgtttttg gtggtgctgt ctgtggcggc gggccctgtc 60  
 tcccagcttg ctcgggcgag cgacgacagc gtcgacagcg tcgaaaccgc gcgtcagcac 120  
 atggagctgg ctatcgaggc tgacgaagag atgcacgagg cctacgaccc tttgttgga 180  
 ttcgttgaga cgtttcggga aatcaaaaaa gctgttgagg aagatgcggc tctgagtaca 240  
 gatgcgatcg accgcgtgtc ccagttcgat ctgggtttccc tcctagatgt catccgagag 300  
 gctgcacaag caaagttcga tctcctcgga cgcctcatta cagacatcgc cagcggaaac 360  
 ggcgaggggtg ccatggctct gatgggagag gaggtgcgt tcattaggcc aaggaggtca 420  
 aagagagggga aaaagactac aactacaacc agttcatcca caagtacgag tacaacgacc 480  
 acgacatcaa ctaccactac taccactacc accactacga ctactactac aactacgaca 540  
 ccaacaacaa ctacaacaac cacaacaact acaccaacaa caacgacaac aaccacaaca 600  
 actacaccaa caacaacgac aacaaccaca acaactacac caacaacaac gacaacaacc 660  
 acaacgccaa ctacaacgac atctacgaca accactacga ctaccacaac tactactaca 720

ccaactacaa caacgacaac cacggaacca acaactacaa caacaaccac ggaaccaacc 780  
 acaactacaa gcacaacgac gactacgaca actacaacga ctacgacacc atctacgacg 840  
 acatccacca ccactaccct cgattag 867

<210> 343  
 <211> 1397  
 <212> DNA  
 <213> Toxoplasma gondii

<220>  
 <221> CDS  
 <222> (238)..(1104)

<400> 343  
 cccattcca gatatccctg ccgctgtggc gcttgaagc gctcctcgac tgcattgacg 60  
 attccgccgt cacaagactt ttttttccct ttgccaacg tcgagaacct ctcacggacg 120  
 agcaaagtct agtgtttggt ttcagtacgg cggtcgtggt ctctgtgtat gactgacctg 180  
 aagaagcaaa gacttcttgc aacgtagaaa cgcaaaggcg cttctttttt gtgcaat 237  
 atg gca gga agg cag gcg gcg ttg ttt ttg gtg gtg ctg tct gtg gcg 285  
 Met Ala Gly Arg Gln Ala Ala Leu Phe Leu Val Val Leu Ser Val Ala  
 1 5 10 15  
 gcg ggc cct gtc tcc cag ctt gct cgg gcg agc gac gac agc gtc gac 333  
 Ala Gly Pro Val Ser Gln Leu Ala Arg Ala Ser Asp Asp Ser Val Asp  
 20 25 30  
 agc gtc gaa acc gcg cgt cag cac atg gag ctg gct atc gag gct gac 381  
 Ser Val Glu Thr Ala Arg Gln His Met Glu Leu Ala Ile Glu Ala Asp  
 35 40 45  
 gaa gag atg cac gag gcc tac gac cct ttg ttg gaa ttc gtt gag acg 429  
 Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr  
 50 55 60  
 ttt cgg gaa atc aaa aaa gct gtt gag gaa gat gcg gct ctg agt aca 477  
 Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr  
 65 70 75 80  
 gat gcg atc gac cgc gtg tcc cag ttc gat ctg gtt tcc ctc cta gat 525  
 Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp  
 85 90 95

gtc atc cga gag gct gca caa gca aag ttc gat ctc ctc gga cgc ctc 573  
 Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu  
 100 105 110

att aca gac atc gcc agc gga atc ggc gag ggt gcc atg gct ctg atg 621  
 Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met  
 115 120 125

gga gag gag gct gcg ttc att agg cca agg agg tca aag aga ggg aaa 669  
 Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys  
 130 135 140

aag act aca act aca acc agt tca tcc aca agt acg agt aca acg acc 717  
 Lys Thr Thr Thr Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Thr  
 145 150 155 160

acg aca tca act acc act act acc act acc acc act acg act act act 765  
 Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 165 170 175

aca act acg aca cca aca aca act aca aca acc aca aca act aca cca 813  
 Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro  
 180 185 190

aca aca acg aca aca acc aca aca act aca cca aca aca acg aca aca 861  
 Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr  
 195 200 205

acc aca aca act aca cca aca aca acg aca aca acc aca acg cca act 909  
 Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr  
 210 215 220

aca acg aca tct acg aca acc act acg act acc aca act act act aca 957  
 Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 225 230 235 240

cca act aca aca acg aca acc acg gaa cca aca act aca aca aca acc 1005  
 Pro Thr Thr Thr Thr Thr Thr Thr Glu Pro Thr Thr Thr Thr Thr Thr  
 245 250 255

acg gaa cca acc aca act aca agc aca acg acg act acg aca act aca 1053  
 Thr Glu Pro Thr Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr  
 260 265 270

acg act acg aca cca tct acg acg aca tcc acc acc act acc ctc gat 1101  
 Thr Thr Thr Thr Pro Ser Thr Thr Thr Ser Thr Thr Thr Thr Thr Leu Asp  
 275 280 285

tag accaaagtgt ttttgccgca cagattgtac atgcatcaaa aaacgcgata 1154

tttctttctc gtttgcgctg gcgtcatctg cgtctgccat ttgaatctgt cagcgctgcc 1214

agctccatag ggcgtgctcc ctgattacgt attgcacga agggatgtct tggcttctac 1274

attggcgaac cgtttttggc actccaaaat ttttattaac acaaagcctt gcactggcct 1334

cattgtctat tcatagaacg ataatgtgtt gacctcgaaa aaaaaaaaaa aaaaaaaaaa 1394

aaa 1397

&lt;210&gt; 344

&lt;211&gt; 288

&lt;212&gt; PRT

&lt;213&gt; Toxoplasma gondii

&lt;400&gt; 344

Met Ala Gly Arg Gln Ala Ala Leu Phe Leu Val Val Leu Ser Val Ala  
1 5 10 15

Ala Gly Pro Val Ser Gln Leu Ala Arg Ala Ser Asp Asp Ser Val Asp  
20 25 30

Ser Val Glu Thr Ala Arg Gln His Met Glu Leu Ala Ile Glu Ala Asp  
35 40 45

Glu Glu Met His Glu Ala Tyr Asp Pro Leu Leu Glu Phe Val Glu Thr  
50 55 60

Phe Arg Glu Ile Lys Lys Ala Val Glu Glu Asp Ala Ala Leu Ser Thr  
65 70 75 80

Asp Ala Ile Asp Arg Val Ser Gln Phe Asp Leu Val Ser Leu Leu Asp  
85 90 95

Val Ile Arg Glu Ala Ala Gln Ala Lys Phe Asp Leu Leu Gly Arg Leu  
100 105 110

Ile Thr Asp Ile Ala Ser Gly Ile Gly Glu Gly Ala Met Ala Leu Met  
115 120 125

Gly Glu Glu Ala Ala Phe Ile Arg Pro Arg Arg Ser Lys Arg Gly Lys  
130 135 140

Lys Thr Thr Thr Thr Thr Ser Ser Ser Thr Ser Thr Ser Thr Thr Thr  
145 150 155 160

Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
165 170 175

Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro  
180 185 190

Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr  
195 200 205

Thr Thr Thr Thr Thr Pro Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Pro Thr  
210 215 220

Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
225 230 235 240

Pro Thr Thr Thr Thr Thr Thr Thr Thr Glu Pro Thr Thr Thr Thr Thr Thr Thr  
245 250 255

Thr Glu Pro Thr Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr  
260 265 270

Thr Thr Thr Thr Pro Ser Thr Thr Thr Ser Thr Thr Thr Thr Thr Thr Leu Asp  
275 280 285

<210> 345

<211> 1397

<212> DNA

<213> Toxoplasma gondii

<400> 345

tttttttttt tttttttttt ttttttcgag gtcaacacat ttcggttcta tgaatagaca 60  
atgaggccag tgcaaggctt tgtgttaata aaaattttgg agtgccaaaa acggttcgcc 120  
aatgtagaag ccaagacatc ccttcgtgca aatacgtaat caggagcac gccctatgga 180  
gctggcagcg ctgacagatt caaatggcag acgcagatga cgccagcgca aacgagaaaag 240  
aaatatcgcg ttttttgatg catgtacaat ctgtgcggca aaaacacttt ggtctaatacg 300  
agggtagtgg tgggtgatgt cgtcgtagat ggtgtcgtag tcgttgtagt tgcgtagtc 360  
gtcgttggtgc ttgtagttgt ggttggttcc gtggttggtg ttgtagttgt tggttccgtg 420

gttggtcggtg ttgtagttgg tgtagtagta gttgtggttag tcgtagtggt tgtcgtagat 480  
gtcggtttag ttggcggtgt ggttggtgtc gttgttggtg gtgtagttgt tgtggttgtt 540  
gtcggttgtg ttggtgtagt tgttggtggt gttgtcggtg ttgttggtgt agttgttgtg 600  
gttggtttag ttgttggtgg tgtcgtagtt gtagtagtag tcgtagtggt ggtagtggta 660  
gtagtggttag ttgatgtcgt ggtcggttga ctcgtaactg tggatgaact ggttgtagtt 720  
gtagtctttt tccctctctt tgacctcctt ggcctaata acgcagcctc ctctcccatc 780  
agagccatgg caccctcgcc gattccgctg gcgatgtctg taatgaggcg tccgaggaga 840  
tcgaactttg cttgtgcagc ctctcggtg acatctagga gggaaaccag atcgaactgg 900  
gacacgcggt cgatcgcac tgtactcaga gccgcattt cctcaacagc ttttttgatt 960  
tcccgaacg tctcaacgaa ttccaacaaa ggtcgtagg cctcgtgcat ctcttcgtca 1020  
gcctcgatag ccagctccat gtgctgacgc gcggtttcga cgctgtcgac gctgtcgctg 1080  
ctcgcccgag caagctggga gacagggccc gccgccacag acagcaccac caaaaacaac 1140  
gccgcctgcc ttcttgccat attgcacaaa aaagaagcgc ctttgcggtt ctacgttgca 1200  
agaagtcttt gcttcttcag gtcagtcata cacagagcca gcgaccgccg tactgaaacc 1260  
aaacactaga ctttgctcgt ccgtgagagg ttctcgacgt tggcaaaagg gaaaaaaaaag 1320  
tcttgtagc gcggaatgct caatgcagtc gaggagcgct tccaggcgcc acagcggcag 1380  
ggatatctgg aatgggg 1397

<210> 346

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 346

caggaaacag ctatgacc

18

<210> 347  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 347  
attaaccctc actaaaggga

20

<210> 348  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 348  
taatacgact cactataggg

20

<210> 349  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 349  
gccagtgtga tggatatctg cag

23

<210> 350  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
Primer



<400> 350  
cgagctcgga tccactag

18

<210> 351  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 351  
ggttctctcc agaggttcat tac

23

<210> 352  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 352  
ggatgcaatg aagagagggc tc

22

<210> 353  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 353  
aactagaagg cacagtcgag gctg

24

<210> 354  
<211> 24  
<212> DNA  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 354

gggaacaaaa gctggagctc cacc

24

&lt;210&gt; 355

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 355

cggacgttgc atgtcagtgg aca

23

&lt;210&gt; 356

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 356

cacgaagctg catgttccag ctag

24

&lt;210&gt; 357

&lt;211&gt; 24

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
Primer

&lt;400&gt; 357

acacttttgt ctaatcgagg gtag

24

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<223> Description of Artificial Sequence: Synthetic  
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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
Primer

<400> 364

ggccacgcgt cgactacttt tttttttttt tttt

34

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/27137

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/30 C07K14/45 C12N15/11 C12N5/10 C07K16/20  
A61K48/00 A61K39/002 A61K39/395 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 516 381 A (MERCK & CO INC) 2 December 1992 see examples 3,4,6 ---	39-42
A	EP 0 687 471 A (BAYER CORPORATION) 20 December 1995 see the whole document ---	1-38
A	EP 0 710 724 A (AKZO NOBEL N. V.) 8 May 1996 see page 7 - page 9 ---	1-38
A	EP 0 700 991 A (BAYER CORPORATION) 13 March 1996 see the whole document ---	1-38
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex

### \* Special categories of cited documents:

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- "P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search

19 April 1999

Date of mailing of the international search report

03/05/1999

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Authorized officer

Mandl, B

# INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/US 98/27137

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 00740 A (UNIV KANSAS) 24 January 1991 see the whole document ---	1-38
X	EMBL database entry TG1932; accession number N82193; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100353 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TGW667; accession number W96667; 19. July 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100354 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TG5911; accession number N61591; 29. Feb. 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100355 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TGAA20976; accession number AA520976; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100356 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TGAA20558; accession number AA520558; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100357 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TGAA32000; accession number AA532000; 24. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100358 see abstract ---	1-7, 10, 12-31
	-/--	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/27137

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL database entry TGAA19977; accession number AA519977; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100359 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TGAA20348; accession number AA520348; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100360 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TG0292; accession number N82029; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100361 see abstract ---	1-7, 10, 21-31
X	EMBL database entry TGAA31653; accession number AA531653; 24. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100362 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TG1673; accession number N82167; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100363 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TG5032; accession number N81503; 13. April 1996; Hehl et al.: 'The WashU-Merck-Stanford-NIH Toxoplasma EST project.' XP002100364 see abstract ---	1-7, 10, 12-31
X	EMBL database entry TGAA20213; accession number AA520213; 17. July 1997; Marra et al.: 'The WashU-Stanford-PAMF-NIH Toxoplasma EST project.' XP002100365 see abstract -----	1-7, 10, 12-31

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 27137

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 9 and 32-38  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority ~~did not~~ invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.



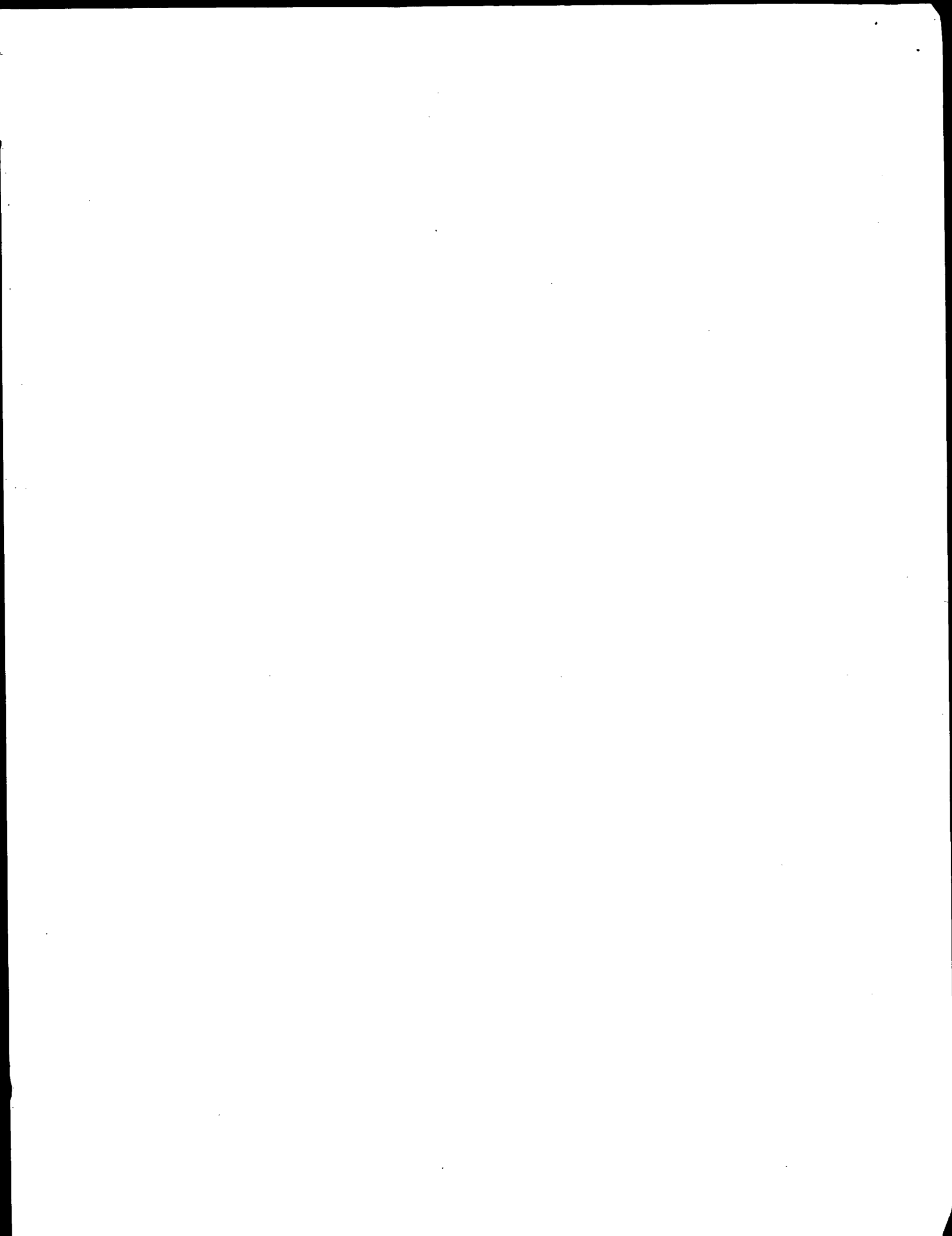
# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/27137

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		AT 157881 T	15-09-1997
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		DE 69031426 T	04-06-1998
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		JP 4507098 T	10-12-1992





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/30, C07K 14/45, C12N 15/11, 5/10, C07K 16/20, A61K 48/00, 39/002, 39/395, C12Q 1/68</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/32633</b>  <b>(43) International Publication Date:</b> 1 July 1999 (01.07.99)
<b>(21) International Application Number:</b> PCT/US98/27137 <b>(22) International Filing Date:</b> 18 December 1998 (18.12.98)  <b>(30) Priority Data:</b> 08/994,825 19 December 1997 (19.12.97) US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b> US 08/994,825 (CIP) Filed on 19 December 1997 (19.12.97)  <b>(71) Applicant (for all designated States except US):</b> HESKA CORPORATION [US/US]; 1613 Prospect Parkway, Fort Collins, CO 80525 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MILHAUSEN, Michael, James [US/US]; 2292 Holyoke Drive, Boulder, CO 80303 (US). LUTZ, Susan, Bektesh [US/US]; 206 W. 12th Street, P.O. Box 417, New England, ND 58647-7210 (US). NG, Ray, K. [US/US]; 412 Alpert Circle, Fort Collins, CO 80525 (US).  <b>(74) Agents:</b> HANLEY, Elizabeth, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> TOXOPLASMA GONDII PROTEINS, CORRESPONDING NUCLEIC ACID MOLECULES, AND USES THEREOF		
<b>(57) Abstract</b>  <p>The present invention relates to immunogenic <i>Toxoplasma gondii</i> proteins, to <i>T. gondii</i> nucleic acid molecules, including those that encode such proteins and to antibodies raised against such proteins. The present invention also includes methods to obtain such proteins, nucleic acid molecules and antibodies. Also included in the present invention are compositions comprising such proteins, nucleic acid molecules and/or antibodies, as well as the use of such compositions to inhibit oocyst shedding by cats due to infection with <i>T. gondii</i>. The present invention also includes the use of certain <i>T. gondii</i>-based antisera to identify such nucleic acid molecules and proteins, as well as nucleic acid molecules and proteins identified by such methods. The present invention also relates to methods for the detection of cysts and oocysts.</p>		

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